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ENDOCRINE ABNORMALITIES ACCOMPANYING HEPATIC CIRRHOSIS AND HEPATOMA*

RICHARD W. BARR, M.D. AND SHELDON C. SOMMERS, M.D.
The Laboratory of Pathology, Massachusetts Memorial Hospitals and the Department of Pathology, Boston University School of Medicine, Boston, Massachusetts

SINCE Silvestrini (1) and Corda (2) described the triad of testicular atrophy, gynecomastia and cirrhosis, a literature of considerable magnitude has appeared concerning endocrine imbalances associated with hepatic damage. Many of the signs and symptoms of cirrhosis have been demonstrated to be due to hormonal changes. Usually these reports have dealt with individual endocrine upsets in certain patients. The purpose of this paper is to consider in a clinicopathologic manner various endocrine glandular alterations observed at autopsy in men and women with cirrhosis, employing pituitary cell counts and including some newly described tissue changes induced by hormones.

METHODS AND MATERIALS

For this study, 100 cases of cirrhosis (involving 70 men and 30 women) were collected from autopsy files of hospitals in the Boston area. There were 67 cases of Laennec's cirrhosis (8 with accompanying hepatoma), 18 of post-necrotic cirrhosis (3 with hepatoma), 8 of biliary cirrhosis, 7 of hemochromatosis (5 with hepatoma), and 1 case of hepatoma without cirrhosis. Accepted criteria for the diagnosis of cirrhosis were employed, and cases difficult to classify were reviewed in consultation with Dr. G. K. Mallory. Endocrine organs and all other available body tissues stained with hematoxylin and eosin were carefully scrutinized histologically. Each study was completed so far as possible by replacing any missing histologic sections with those from the wet preserved stock tissues. Pituitary differential cell counts were performed by the method of Rasmussen

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(3) with Pearse's (4) periodic-acid Schiff trichrome stain, on an average of 10,000 cells per case.

RESULTS

The major endocrine glandular and target-organ alterations observed are listed in Table 1. They have been compared with the corresponding autopsy findings in unselected non-cancer patients of comparable age, and they have also been considered by age and sex groups.

Cirrhotic younger women. Among 30 cirrhotic women, 12 were between 23 and 48 years old at the time of death. In 6 the cirrhosis was relatively slight, and in 6 it was moderate or severe. As a group, these women manifested few, if any, important alterations of the endocrine glands. The ovary and uterus appeared inactive in 5 of 9 available cases, as frequently occurs in various wasting diseases. An estrogenic effect (5) upon the cervical epithelium was evident in 4 of 6 cases, including 2 of 9 cases in which the ovaries showed cortical stromal hyperplasia (6). In 10 cases breast tissue was reviewed microscopically; in 2 the findings were negative, in 7 there

TABLE 1. SALIENT PATHOLOGIC ENDOCRINE ALTERATIONS IN 70 MEN AND 30 WOMEN WITH CIRRHOSIS, AND IN A GROUP OF CONTROLS

	In cirrhosis	In controls
Spermatic tubular tunica thickened	58/64*(91%)	88/168 (52%)
Estrogenized cervix uteri	11/13 (85%)	34/ 53 (64%)
Spermatogenesis diminished	42/64 (74%)	113/169 (67%)
Testicular spermatogenic hypertrophy	34/50 (68%)	48/148 (32%)
Adrenocortical nodularity	65/98 (64%)	169/454 (37%)
Rete-testis hyperplasia	12/19 (63%)	—
Ovarian stromal hyperplasia	16/26 (62%)	98/265 (37%)
Adrenal cortex narrow	60/98 (61%)	100/454 (22%)
Testicular interstitial-cell atrophy	38/64 (59%)	96/169 (57%)
Prostatic atrophy	39/66 (59%)	31/223 (14%)
Endometrial hyperplasia	14/25 (56%)	67/248 (27%)
Hyalinized pancreatic islets (diabetics)	9/17 (53%)	20/ 76 (26%)
Pituitary basophil hyperplasia	24/48 (50%)	31/238 (13%)
Mammary adenofibrosis	11/24 (46%)	22/ 49 (45%)
Pancreatic fibrosis	45/99 (46%)	6/443 (1%)
Thyroid atrophy	29/94 (31%)	74/408 (18%)
Prostatic squamous metaplasia	16/66 (24%)	3/223 (1%)
Pancreatic islet hyperplasia	23/99 (23%)	24/443 (5%)
Thyroid nodularity	21/94 (22%)	31/408 (8%)
Pancreatic duct hyperplasia	17/99 (17%)	33/443 (7%)
Pancreatitis	15/99 (15%)	6/443 (7%)
Parathyroid hyperplasia	6/44 (14%)	24/154 (16%)

* In 58 of 64 cases.

was adenofibrosis, and in 1 chronic cystic mastitis (7). Thus, although at death other tissue evidences of estrogenic activities were found in less than half of these cirrhotic women in the childbearing age, mammary target-organ indications of prolonged or excessive estrogen stimulation or both were found in four-fifths of the collected cases.

Almost all the adrenal cortices were unusually narrow, and the zona fasciculata was nodular in all 4 women over 40 years old. The thyroid gland was slightly atrophied in this somewhat older group. There was no significant frequency of pancreatic alterations.

Cirrhotic older women. In 18 postmenopausal cirrhotic women there was evidence of estrogen stimulation of the cervical epithelium, or of endometrial hyperplasia in 14 of the 16 available cases (Figs. 1 and 2). The ovarian cortical stroma was hyperplastic in 11 of 15 older women (Fig. 3). Breasts were adenofibrotic in 2, and cystic in 3 of the 12 available cases. Three of the 7 otherwise normal breast specimens showed concentric periductal hyaline bands recently recognized as fairly common among diabetic women (8).

Adrenal cortices were unusually narrow in 7 of the 17 older women (Fig. 4), but contained hyperplastic nodular thickenings of the zona fasciculata in the 2 oldest patients, aged 75 and 79 years. Ten patients had nodular adrenal cortices. Thyroid tissue was nodular in 9 women (including 2 with adenomatous goiter) and was atrophic in 2 of the group of 16 glands examined. Sections of the pancreas usually showed either inflammation or fibrosis. Islets of Langerhans were hyperplastic in 4 cases and hyalinized in 2. The pancreatic ducts were hyperplastic in 2 cases.

One 48-year-old woman had cancer of the breast, and malignant hepatomas were found at death in 2 patients aged 52 and 64 years.

Pituitary differential cell counts in 10 cirrhotic women varied for the most part within the expected normal limits of all cell types, except for hypertrophic amphophils and hyaline basophils (9) (Table 2). The average of 1.4 per cent for the hypertrophic amphophils represented a borderline increase. An almost complete absence of Crooke's hyaline basophil cells suggested that the cortisone output in these cirrhotic women was below average. Unlike previous observations in women without cirrhosis, ovarian stromal hyperplasia was not found to be regularly associated with increased percentages of normal so-called gonadotropic basophils (7).

Cirrhotic younger men. In 16 of 70 men, death had occurred before 50 years of age (range, 34 to 49 years). In only 4 of 13 studied was there active spermatogenesis, and in the remainder spermatozoa were few or absent. In 11 of 13 testes the tubular lamina propria was thickened, as a further indication of atrophy, and in 4 testes there was local complete hyalinization of tubules.

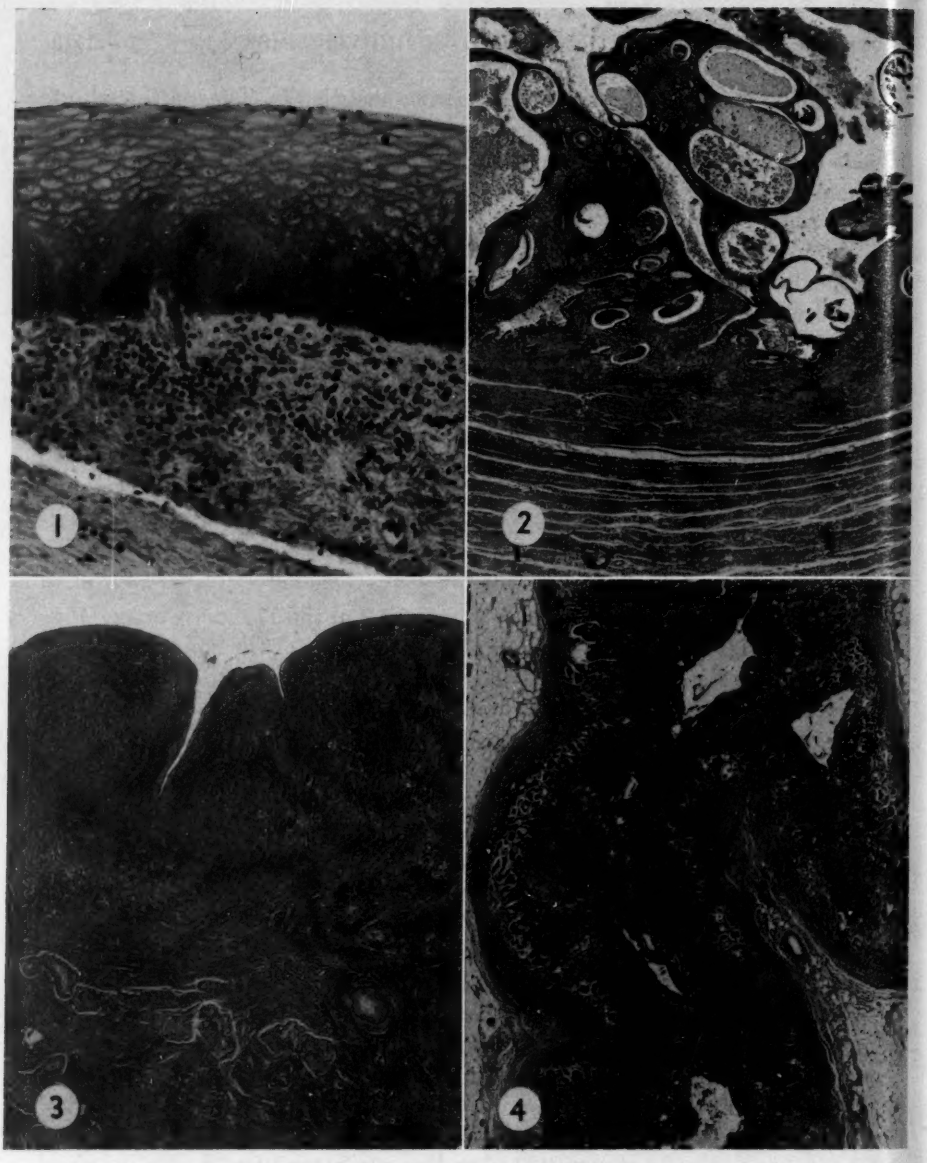

FIG. 1. Estrogen effect on the uterine cervical epithelium, as observed in all of the specimens available from the postmenopausal women with cirrhosis. Hematoxylin-eosin, ×250.

FIG. 2. Polypoid cystic endometrial hyperplasia, from a 64-year-old woman with cirrhosis, malignant hepatoma and ovarian stromal hyperplasia. Hematoxylin-eosin, ×25.

FIG. 3. Nodular hyperplasia of the ovarian cortical stroma, from a 68-year-old woman with biliary cirrhosis. An estrogenic effect was evident in the uterine cervix. Hematoxylin-eosin, ×25.

FIG. 4. Narrow adrenal cortex with nodularity of the zona fasciculata and a thickened fibrous capsule, from the case represented in Fig. 2. Hematoxylin-eosin, ×25.

A recently reported criterion for estrogenic effect upon testicular tubules consists in finding increased numbers of abnormally hypertrophied and hydropic partly matured spermatogenic cells (10). When the average number of such cells per tubule exceeds 3 in a count of 10 cross-sections of tubules, increased estrogenic stimulation may be inferred. Of 11 cases with persisting spermatogenic elements, 8 had significantly increased indices of

TABLE 2. AVERAGE PITUITARY CELL COUNTS IN CIRRHOSIS, IN HEPATOMA AND IN CONTROL CASES

	Avge. age	No. of cells counted	Differential count (%)					
			Ac	Bas	Amp	Chr	HA	HB
10 Cirrhotic women	46	10,573	37.1 ±7.0	17.0 ±5.4	24.2 ±4.7	20.3 ±2.0	1.4 ±0.7	0*
11 Cirrhotic men	59	10,190	34.8 ±5.5	21.7 ±4.6	21.6 ±4.9	20.3 ±2.0	1.5 ±0.6	0†
10 Hepatoma patients**	67	10,292	29.9 ±6.6	25.2 ±6.6	21.9 ±6.7	21.1 ±2.1	1.8 ±0.5	0
10 Control women	45	10,470	37.4 ±5.3	17.6 ±2.8	22.4 ±5.5	21.5 ±3.1	0.9 ±0.1	0.1‡
10 Control men	50	10,136	40.8 ±7.0	17.8 ±2.4	19.1 ±5.9	21.5 ±1.3	0.8 ±0.4	0.1‡

Ac = acidophils; Bas = basophils; Amp = amphophils; Chr = chromophobes; HA = hypertrophic amphophils; HB = hyaline basophils.

* One patient had <0.1% hyaline basophils.

† Four patients had up to 0.1% hyaline basophils.

** Nine men; 1 woman.

‡ Three patients had <0.1% hyaline basophils.

spermatogenic hypertrophy (I.S.H.), ranging between 3.1 and 4.1 (Fig. 5). Interstitial cells of Leydig were usually numerous, although sometimes rather shrunken. In all 3 cases in which the testicular rete was examined microscopically (Fig. 6), further estrogenic influence upon the genital tract was observed in the form of papillary hyperplastic infoldings of the epithelium of testicular rete tubules, somewhat similar to the changes found in papillary ovarian cystadenomas.

The prostate gland was grossly and microscopically unaltered in 8 of the 16 younger cirrhotic men, and in 3 showed only the simple atrophy commonly found in wasting diseases. In the other 5 young men there was a sclerotic fibrous stromal overgrowth of subcapsular portions of the pros-

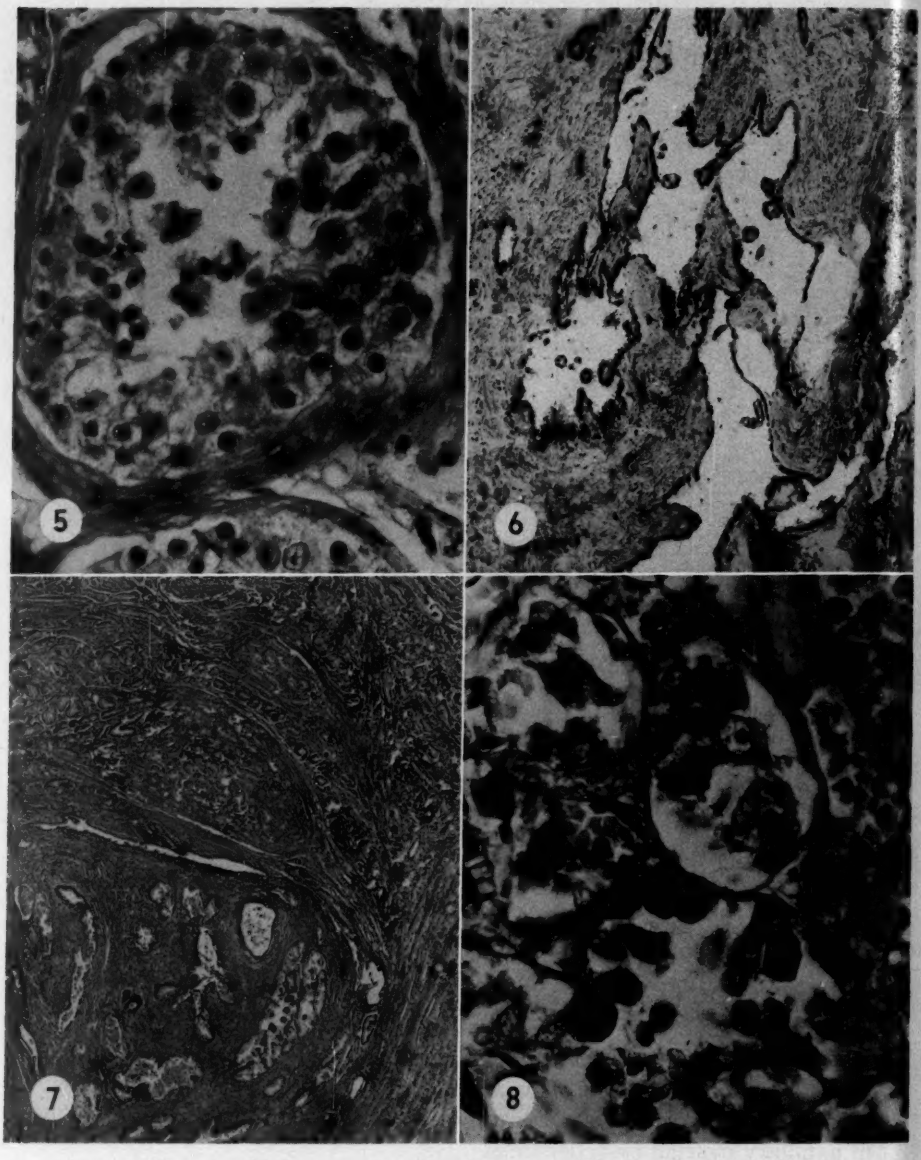

FIG. 5. Hypertrophied spermatogenic cells, some with clear hydropic cytoplasm, located two or more cell layers above the tunica propria—from the testis of a 62-year-old man with hemochromatosis. The index of spermatogenic hypertrophy (I.S.H.) was 3.9 (see text). Hematoxylin-eosin, ×250.

FIG. 6. Papillary overgrowth of testicular rete tubules, from a 62-year-old man with

tate, identical with that described as "sclerotic atrophy" by Franks (11) and ascribed by him to estrogen.

The adrenal cortex was unusually narrow in 9 men, of normal appearance in 5, and contained an adenoma of the zona fasciculata in 1. The thyroid gland appeared normal in 12, atrophied in 3 and abnormally nodular in 1 case. Pancreatic islets appeared to be hyperplastic in 2 cases.

Cirrhotic older men. There were 54 deaths from cirrhosis in men beyond 50 years of age—the largest subgroup studied. Spermatogenesis was active in 18 of 50 available cases, slight in 18, and absent in 14. The lamina propria of the tubules was thickened in 42 of the 50 cases. Interstitial cells were few in 36 men, and fairly abundant in 14. In 39 cases in which a count was made, abnormally increased numbers of hypertrophied spermatogonia were observed in 26 men (67 per cent) with significant numbers of spermatogenic cells, and the indexes of spermatogenic hypertrophy (I.S.H.) ranged up to 4.8. Rete tubules were hyperplastic in 9 of the 16 cases with demonstrable rete structures.

In a group of 51 older men whose prostatic tissue was analyzed, 11 prostate glands appeared normal and 17 demonstrated simple atrophy. There were 13 cases of sclerotic atrophy, 6 of benign prostatic hypertrophy and superimposed sclerotic atrophy, and 2 of benign hypertrophy and subsequent simple atrophy. Primary prostatic carcinomas completely replaced 2 glands, in 1 instance associated with sclerotic atrophy and in 1 instance with benign hypertrophy and sclerotic atrophy (Fig. 7). Squamous metaplasia of the prostatic epithelium was observed in 15 of 49 cases studied (31 per cent).

Adrenal cortices appeared narrow in 31 of 54 available cases, including 21 with local nodularity. In the remaining 23 cases, the adrenal cortex was nodular in 18. Of 47 thyroid glands studied, atrophy was found in 8, and nodularity in 4. Pancreatic islets were hyperplastic in 7 cases, and an islet adenoma was present in 1. Hyalinized islets were seen in 6, and squamous metaplasia of the ducts in 6. Changes in the exocrine pancreas, involving atrophy, fibrosis or inflammation, were common.

Laennec's cirrhosis. The index of spermatogenic hypertrophy (I.S.H.) was 4.0. Hematoxylin-eosin, ×25.

FIG. 7. Section from prostate of an 83-year-old man with post-necrotic cirrhosis. In the lower area is shown nodular benign prostatic hypertrophy, with sclerotic atrophy of the glands and overgrowth of fibrous stroma. Above is prostatic adenocarcinoma. Hematoxylin-eosin, ×25.

FIG. 8. A pituitary hypertrophic amphophil cell is shown in the right upper quadrant, with a large nucleolus and indistinct cytoplasm. Section is from the pituitary of a 52-year-old man with hemochromatosis and malignant hepatoma. The differential count of pituitary cells included 2.7% hypertrophic amphophils. The very dark cells are PAS-positive basophils (33.1%); the medium gray cells are acidophils (16.4%); and the paler reticulated cells are amphophils (26.4%). Pearse stain, ×475.

Differential counts of pituitary cells in 11 male cirrhotics (Table 2) showed a slight average increase (sometimes individually significant) in the percentages of normally granulated basophils and hypertrophic amphophils. There was a suggestive decrease of Crooke's hyaline basophils to nearly zero. These counts, made without prior knowledge of the clinical diagnosis or of the patient's sex, indicate that a hyperactive pituitary gonadotropic-cell response to estrogen often characterizes cirrhosis in men. Although 3 men with altered pituitary cell counts had benign prostatic hypertrophy, the possibility appears unlikely that an unrelated endocrine imbalance chiefly accounted for the changes, since the most abnormal counts were found in association with prostatic atrophy.

Hepatoma. All 17 cases occurred in persons over 50 years old, and all but 2 occurred in men. At the time of death 2 patients were in the 50–59 year age group, 7 were in the 60–69 group, and 8 were aged 70 or over. Ten malignant neoplasms of the liver had not metastasized, 2 had metastasized locally and 5 had reached the lungs and lymph nodes.

Endocrine changes in the men included 7 cases of sclerotic atrophy of the prostate—2 superimposed on benign hypertrophy. All 4 prostatic carcinomas observed in cirrhotic men were accompanied by hepatomas. Testicular indices of spermatogenic hypertrophy were abnormally high in 9 of the 12 cases with persisting spermatogonia, and in 3 others there was complete atrophy of the testes. In 5 cases the adrenal cortices were narrow and the thyroid was atrophic. Pancreatic ducts and islets were hyperplastic in 4 cases. In the 2 women there were some evidences of estrogenic stimulation, besides narrow adrenal cortices and altered structure of the thyroid and pancreas.

Differential counts of pituitary cells in 10 cases of hepatoma (Table 2) differed from those in cirrhosis by the higher average percentages of gonadotropic basophils and hypertrophic amphophils, in association with a decrease in acidophils (9). Due to considerable variation within the relatively small groups, the differences were not statistically significant when using the *t*-test to compare mean values in hepatoma, cirrhosis and control groups (12). Changes in the basophil and amphophil cell counts were comparable to those found in association with other invasive internal cancers previously studied. The four highest percentages of hypertrophic amphophils were found in the presence of metastases (2.7, 2.4, 2.1 and 1.8 per cent), although higher values have characterized other more widespread cancers (Fig. 8).

Endocrine correlations. Cirrhosis in women was accompanied by uterine, breast or ovarian stigmata of estrogenic secretion in 85 per cent of cases (22 among 26), compared to about 64 per cent of controls. Unusually narrow adrenal cortices were commonly observed (in 18 of 29 cases, or 62 per cent, including all but 1 premenopausal case). The zona fasciculata, where

cortisone-type steroids originate, was particularly affected. Other endocrine glands were usually not altered.

Two thirds of both younger and older men dying with cirrhosis had testicular changes presumably due to a relative preponderance of estrogen. If the observations of de la Balze and co-workers (13) regarding progressive fibrosis and hyalinization of the testicular tubules during estrogen therapy be acceptable, over 90 per cent of the cirrhotic men had abnormalities attributable to estrogen, compared to about 50 per cent of the controls. The incidence of benign prostatic hypertrophy was low; it occurred in only 16 per cent of men over 50 years of age. Sclerotic atrophy of the prostate was observed in 24 of the 67 total cases (36 per cent), and hyperplasia of the rete tubules in 12 of 19 cases (63 per cent). All these findings favored uninhibited estrogenic activity. Hepatomas were found in 15 of the older men, along with 4 prostatic carcinomas, 1 pancreatic adenocarcinoma, 1 epidermoid carcinoma of esophagus and 1 early bronchiolar carcinoma. Among 60 male cirrhotic patients over 48 years old, 30 per cent had cancer. In 40 of 69 cirrhotic men (58 per cent) the adrenal cortices were unusually narrow.

Judged on the basis of the endocrine alterations, the patients with hepatoma had responded more actively and in a more generalized manner to the continuous effects of estrogen. Pituitary-adrenal alterations also were more evident in cases of hepatoma than in cases of cirrhosis. In the hepatomatous patients, there is a suggestion that estrogen actually may have been a carcinogen or a co-carcinogen in the liver, and at times simultaneously in the prostate (14, 15). The cases in women were too few for analysis.

Clinico-pathologic aspects. The series of 100 cases was not unusual from the viewpoint of clinical, anamnestic and general pathologic findings, as shown in Table 3. The causes of death in 64 instances were listed as follows: 25 from hepatic coma, 16 from bleeding esophageal varices, 8 from bronchopneumonia, 8 from cardio-respiratory failure, 2 from peptic ulcer complications, 2 from cerebral hemorrhage, and 1 each from intestinal obstruction, tuberculosis, and lower nephron nephrosis.

Judged pathologically, the cirrhosis was severe in 39, moderate in 39, and mild in 22 of the 100 cases. Regenerative hyperplasia of the bile duct was twice as frequent in men, whereas regenerative nodules in the liver cells were equally common in women. Fibrocongestive splenomegaly was present in 54 of 92 cases (59 per cent). The severity of the cirrhosis and its secondary effects on the spleen were less marked in the women; thus 57 of 70 men (82 per cent) had moderate or advanced cirrhosis, compared to 21 of 30 women (70 per cent). Fibrocongestive splenic alterations were found in 65 per cent of the men and 42 per cent of the women.

A generalized hyaline thickening of the pulmonary arterioles and small

arteries was noted in 50 of the 100 cirrhotics, varying from slight in 20 cases to moderate or marked in 30 cases. Occasionally hyaline material was seen in the vessel walls unaccompanied by vascular narrowing or thickening. In 21 cases, a peculiar similar sclerosis was observed in the renal glomeruli, somewhat resembling that in toxemia of pregnancy. The basement membrane of the glomeruli was uniformly thickened and hyalinized.

TABLE 3. MISCELLANEOUS CLINICAL AND PATHOLOGIC OBSERVATIONS IN 100 CASES OF CIRRHOSIS

	Incidence
Cephalin flocculation abnormal	56/ 65*(86%)
Hypoproteinemia	36/ 43 (83%)
Reversed A/G ratio	34/ 43 (79%)
Ascites	79/100 (79%)
Jaundice	78/100 (78%)
Bone marrow hyperplasia	49/ 80 (61%)
Alcoholism with Laennec's cirrhosis†	44/ 72 (61%)
Fibrocongestive splenomegaly	54/ 92 (59%)
Pulmonary arteriolar sclerosis	50/100 (50%)
Cutaneous telangiectases	36/ 79 (45%)
Esophageal varices	36/100 (36%)
Married and childless†	9/ 26 (35%)
Anemia	17/ 51 (33%)
Gastritis	18/ 60 (30%)
Hepatic coma	25/100 (25%)
Hepatitis with post-necrotic cirrhosis†	16/ 72 (22%)
Glomerular sclerosis	21/100 (21%)
Diabetes mellitus	17/100 (17%)
Peptic ulcer (gastric 6, duodenal 9)	15/100 (15%)
Bleeding varices	12/100 (12%)
Gynecomastia	5/ 58 (9%)
Syphilis†	5/ 60 (8%)

* In 56 of 65 cases.
† By history.

Often material resembling fibrin was seen in layers along the glomerular capillaries.

Results of clinical laboratory tests of hepatic function were usually abnormal. Cephalin flocculation was the most consistently performed liver function test; 56 of the 65 available determinations showed significant elevation of values. In over 80 per cent of the cases there was hypoproteinemia with marked reversal of the albumin-globulin ratio. No significant differences were observed between cases of hepatoma and cases of advanced

cirrhosis. Anemia was present in a third of the patients, and there was evidence of polycythemia in 3.

Over half the urines contained more than 2-plus albumin. Fifty-seven per cent of the 21 patients with glomerular sclerosis had significant albuminuria, compared to 59 per cent of the patients without this lesion.

DISCUSSION

On the basis of analysis of the present series of cases, there is a considerable amount of individual variation in the endocrine abnormalities associated with cirrhosis. However, despite histologic differences, the overall indications are that hepatic cirrhosis characteristically is accompanied by some stigmata of estrogenic hormonal activity. The data on tissue pathology have permitted only an inference of estrogenic effects, but methods involving assay of urinary estrogens suffer equally from the limitations of indirectness.

Eighty-five per cent of the women and 91 per cent of the men with cirrhosis manifested some indications of common estrogenic hormonal influences, compared to about 50 per cent of the control subjects. It could be inferred that estrogen was the cause of the excessive stimulation of the breast and endometrium observed in about half the women (6, 7), and of the structural alterations in the prostate and in the testicular tubules in about two-thirds of the men with cirrhosis (11, 13). Of the various other endocrine abnormalities listed in Table 1, only the adrenal, pancreatic and parathyroid changes were not correlated with those previously observed in estrogenized persons and in experimental animals (16–18).

Pituitary cytologic changes differed between the female and male cirrhotic groups. Free estrogen, rather than any conjugated derivative, usually is believed to be the agent involved in estrogenic alteration of pituitary gonadotropic activity (19). In the female cirrhotic subjects who were completely studied, there was no significant increase in pituitary activity; therefore, free estrogen levels were probably within normal limits. In several cirrhotic men, either because of more severe liver damage, greater estrogen production or less hepatic conjugation (20), hyperplasia of the pituitary basophilic cells had developed, due to stimulation by excessive free estrogen. In over half of the male cirrhotic subjects, the excess estrogen appeared to rise to levels comparable to those achieved with stilbestrol therapy for prostatic cancer, as judged by effects on the prostate (21). Estrogen in circulation exceeded the mildly elevated levels associated with growth of hyperplastic nodules of the prostate (10).

Also of interest was the predominance of narrow and nodular adrenal cortices in patients with cirrhosis, and the reduced number of special pituitary cells indicative of a response to cortisone (9). Either the primary

or the secondary changes associated with subnormal cortisone secretion were found in about two thirds of the cirrhotic patients, but in less than 25 per cent of the control subjects. Clinical evidence of adrenocortical hypofunction in Laennec's cirrhosis has already been reported (22).

In patients with hepatoma, compared to those with cirrhosis or non-cancerous conditions (controls), there was an increased frequency of pituitary basophilic hyperplasia, hypertrophic amphophil cells, and prostatic carcinoma. This suggested that in human males, excess estrogen may be co-carcinogenic for liver tissue (23). The possible role of estrogen in the development of prostatic cancer is discussed elsewhere (24).

SUMMARY

One hundred cases of hepatic cirrhosis, including 17 hepatomas, were collected from the autopsy files of Boston hospitals and analyzed particularly for endocrine abnormalities. Allowing for individual variations, in 85 per cent of the 30 women and in over 90 per cent of the 70 men there was evidence of estrogenic effects. It was inferred that there was an excess of estrogen in circulation in about half of the women and two thirds of the men with cirrhosis. Pituitary basophilism of the gonadotropic type occurred chiefly in men with cirrhosis and hepatoma. The data suggest that estrogen may have been co-carcinogenic in the cirrhotic men. Pituitary and adrenocortical morphology was such as to indicate subnormal productions of cortisone. Distinctive pulmonary arteriolosclerotic and glomerulosclerotic lesions were found in association with cirrhosis.

Acknowledgments

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A QUANTITATIVE STUDY OF THE EFFECT OF THYROTROPIN UPON THE THYROIDAL SECRETION RATE IN EUTHYROID AND THYROTOXIC SUBJECTS*

MONTE A. GREER, M.D.† AND HERBERT F. SHULL

Radioisotope Service, Veterans Administration Hospital, Long Beach, and the Department of Medicine, University of California School of Medicine, Los Angeles, California

ALTHOUGH previous investigations have demonstrated that the thyroid gland of both euthyroid and thyrotoxic patients responds to administration of exogenous thyrotropin (1–7), the existence of differences in the nature of this response has not been explored fully.

It was therefore considered of interest to make a quantitative study in normal and hyperthyroid subjects of the effect of varying doses of thyrotropin. In most thyrotoxic patients the thyroidal uptake of I^{131} is so high initially that the detection of any stimulation by means of this measurement is difficult. Of the parameters available for assessing thyroid function, we therefore employed the thyroidal secretion rate, as measured by the release of radioactive iodine from the thyroid gland, believing this to be the most sensitive. Use of the rate of thyroidal secretion as a parameter also obviates the necessity of giving repeated doses of radioisotope. In addition, corollary studies were made of changes in the serum protein-bound stable iodine and protein-bound I^{131} levels.

MATERIALS AND METHODS

Twenty euthyroid human subjects ranging in age from 18 to 77, and 10 thyrotoxic subjects ranging in age from 22 to 62, were employed for this study. Only 1 euthyroid subject and 2 thyrotoxic subjects were female. The patients were judged to be unequivocally either euthyroid or thyrotoxic by the usual clinical and laboratory criteria. All thyrotoxic patients had what was clinically classified as a diffuse goiter; all but 1 had varying degrees of exophthalmos.

Determination of the thyroidal secretion rate was made, as previously described (7), according to a modification of the method of Goldsmith *et al.* (2). Beginning twenty-four

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† Present address: Department of Medicine, University of Oregon Medical School, Portland, Oregon.

hours after oral administration of 100 to 300 microcuries of I^{131}, the radioactivity of the thyroid gland was determined at the same time daily by means of a collimated scintillation counter at a distance of 20 cm. from the skin surface. At this distance the counter had a sensitivity of 420 cpm/μc. I^{131} and a background of 120 cpm. The daily counts were plotted on semilogarithmic paper after correction for physical decay. Beginning with the first count, the patients received 30–60 mg. of methimazole every eight hours to block the reaccumulation in the thyroid of I^{131} liberated from the breakdown of endogenously synthesized labelled thyroid hormone. This dosage of methimazole has been found adequate to block almost completely the organic binding of iodine by the thyroid (11). The method thus permitted an accurate reflection of the rate of secretion of radioiodine from the gland. As previously reported (7–9), this rate was found to be an exponential process. The concentration of stable protein-bound iodine in the serum was determined by a modification of Barker's (10) method and in most cases was measured two to three times weekly during the period of study. The concentration of protein-bound I^{131} was determined by measuring the radioactivity of the washed Somogyi precipitate of 2 ml. of serum in a scintillation well-counter which had a sensitivity of 800,000 cpm/μc. I^{131} and a background of 300 cpm.

Thyrotropin (Armour's "Thytropar," lots P2308 and M2105) was administered intramuscularly once daily at 2:00 P.M., thyroidal counting being performed at 1:00 P.M. Unless otherwise specified, each given dosage was administered for a period of three days. Dosage ranged from 0.3 to 100 U.S.P. units daily and was usually increased every three days, beginning with the smallest dose for each patient. Thyrotropin was not begun until several days after the initiation of each study, in order to permit an evaluation of the initial rate of secretion.

For the purpose of simplicity, the secretion rate is arbitrarily expressed as the biologic half-life in days of the radioactivity in the gland during each period of treatment.

RESULTS

The data are summarized in Tables 1 and 2 and Figures 1–7. The mean initial thyroidal secretion rate of the euthyroid patients, expressed in terms of biologic half-life of thyroidal I^{131}, was 30.5 days (range 7.0 to 164.0 days) and that of the hyperthyroid patients was 10.4 days (range 3.75 to 18.0 days). All of the euthyroid patients and all but 1 of the thyrotoxic patients exhibited an increased secretion rate in response to administration of thyrotropin.

In only 1 of the 6 euthyroid patients given 0.3 unit of thyrotropin was there an increased thyroidal secretion rate. In 7 of the 11 given 1 unit of thyrotropin, a response was obtained. In those receiving 3 or more units daily, there was an accelerated loss of radioiodine from the thyroid.

A somewhat larger amount of thyrotropin was required in the thyrotoxic subjects. None of 6 responded to 1 unit daily; 3 of 10 responded to 3 units daily; 6 of 10 responded to 10 units daily; and all but 1 responded to 30 units daily.

There was no definite qualitative difference between the response of the euthyroid and the response of the thyrotoxic patients. In general, however, the difference in slope between the initial (control) secretion rate and that

TABLE 1. CHANGES PRODUCED IN THYROIDAL SECRETION RATE AND SERUM PROTEIN-BOUND IODINE LEVEL BY INCREASING DOSES OF THYROTROPIN IN EUTHYROID PATIENTS

Patient	Age (yrs.)	Initial control level*	Thyrotropin† (units per day)							Days after last inj. of thyrotropin	
			0.3	1	3	10	30	50	100	Three	Seven
L.H.	42	24.0 4.2	24.0 4.2	8.0 5.0	5.5 6.0	3.5 6.8	— —	— —	— —	∞ 6.0	∞ 4.4
E.S.	47	43.0 4.8	43.0 5.0	12.5 6.4	8.3 7.4	8.3 7.6	— —	— —	— —	∞ —	∞ —
O.M.	51	16.5 5.4	16.5 5.6	16.5 7.4	8.0 8.0	5.5 8.8	— —	— —	— —	∞ —	∞ —
T.G.	51	28.0 5.5	16.0 7.4	13.0 8.4	8.0 9.8	5.5 12.6	— —	— —	— —	∞ 9.4	∞ 5.2
F.Z.	32	18.2 8.2	18.2 8.4	18.2 9.6	5.0 10.8	5.0 11.0	— —	— —	— —	∞ 9.6	∞ 5.8
T.S.	26	31.0 4.4	31.0 5.8	31.0 8.9	9.0 10.4	9.0 12.6	— —	— —	— —	∞ 10.4	∞ —
W.H.	69	7.0 5.0	— —	4.2 5.2	4.2 4.8	4.0 —	— —	— —	— —	350.0 —	350.0 —
T.B.	28	8.5 5.5	— —	5.0 5.2	3.5 5.4	3.0 5.3	— —	— —	— —	∞ 3.8	∞ 2.8
E.J.S.	29	18.0 5.4	— —	8.0 6.4	6.2 8.0	5.0 —	— —	— —	— —	— —	— —
T.Sj.	29	29.0 5.6	— —	29.0 6.0	12.0 —	— —	— —	— —	— —	— —	— —
L.McD.	35	28.5 6.0	— —	10.5 —	6.5 —	4.5 —	— —	— —	— —	∞ —	∞ —
J.L.	60	16.0 6.9	— —	16.0 —	10.0 —	10.0 —	10.0 —	10.0 —	— —	— —	— —
L.Mo.	20	13.0 7.5	— —	— —	— —	7.0 —	7.0 —	7.0 —	7.0 —	— —	— —
H.J.	77	15.0 6.0	— —	— —	— —	5.0 11.2	— —	— —	— —	18.0 8.0	18.0 —
R.N.	38	57.0 5.8	— —	— —	— —	9.5 —	— —	— —	— —	∞ —	∞ —
S.B.	41	15.0 6.3	— —	— —	— —	3.7 —	— —	— —	— —	580.0 —	580.0 —
A.B.	18	164.0 6.1	— —	— —	— —	8.0 —	— —	— —	— —	420.0 —	420.0 —
E.G.	51	11.0 7.5	— —	— —	— —	4.2 —	— —	— —	— —	230.0 —	230.0 —
R.S.	19	41.0 5.9	— —	— —	— —	4.0 —	— —	— —	— —	∞ —	∞ —
L.S.	44	26.0 4.9	— —	— —	— —	4.5 —	— —	— —	— —	— —	— —

* For each patient, the upper line is the thyroidal secretion rate expressed as the biologic half-life of thyroidal I^{131} in days and the lower line is the level of the serum protein-bound iodine in μg./100 ml.

† Thyrotropin expressed as Armour U.S.P. units.

TABLE 2. CHANGES PRODUCED IN THYROIDAL SECRETION RATE AND SERUM PROTEIN-BOUND IODINE LEVEL BY INCREASING DOSES OF THYROTROPIN IN THYROTOXIC PATIENTS

Patient	Age (yrs.)	Initial control levels*	Thyrotropin† (units per day)					Days after last inj. of thyrotropin	
			1	3	10	30	100	Three	Seven
L.Sy.	39	13.0 14.1	13.0 —	13.0 14.2	13.0 13.8	6.0 11.8	— —	∞ 10.8	6.0 10.0
T.L.	53	11.0 8.6	11.0 8.8	11.0 8.8	11.0 8.8	10.0 9.2	— —	29.0 8.2	29.0 7.0
C.H.C.	62	3.8 17.9	3.8 15.8	3.8 13.8	3.0 12.0	3.0 9.2	— —	31.0 5.4	5.0 3.8
R.J.W.	28	16.0 9.8	16.0 8.6	16.0 10.8	10.0 11.4	10.0 11.4	— —	420.0 7.8	420.0 6.5
J.T.	43	5.5 11.6	5.5 —	5.5 —	2.5 —	2.5 —	— —	23.0 —	23.0 —
E.M.C.	23	14.0 13.2	14.0 —	14.0 —	14.0 —	7.0 —	7.0 —	— —	— —
P.G.	42	18.0 12.5	— —	9.0 16.6	6.0 19.5	0.0 15.8	— —	38.0 13.4	38.0 10.0
J.K.	55	5.0 11.2	— —	3.0 12.8	3.0 12.8	3.0 10.8	— —	21.0 9.8	21.0 6.8
E.M.	50	7.0 14.0	— —	7.0 12.8	7.0 12.5	7.0 12.0	— —	7.0 10.5	7.0 9.2
V.C.	22	11.0 9.2	— —	6.0 15.2	6.0 15.0	6.0 —	— —	— —	— —

* For each patient, the upper line is the thyroidal secretion rate expressed as the biologic half-life of thyroidal I^{131} in days and the lower line is the level of the serum protein-bound iodine in μg./100 ml.

† Thyrotropin expressed as Armour U.S.P. units.

during administration of thyrotropin was not as striking in the thyrotoxic as in the euthyroid patients. This might have been expected because of the considerably faster initial rates in the thyrotoxic patients.

By extrapolation it could be calculated that the response to thyrotropin began very shortly after injection in almost all subjects, once an adequate dosage level had been reached. In a few, however, the response seemed to be delayed until more than one daily injection had been given. The increased thyroidal secretion rate resulting from administration of thyrotropin persisted until twenty-four hours after the last injection, at which point the rate of loss from the thyroid was sharply reduced. This was true for both the euthyroid and thyrotoxic patients who responded. In only 1 of the euthyroid patients, H.J., was escape from the post-thyrotropin inhibition seen during the period of study. In 2 of the thyrotoxic patients the decreased rate of I^{131} release persisted for only a few days and a resumption

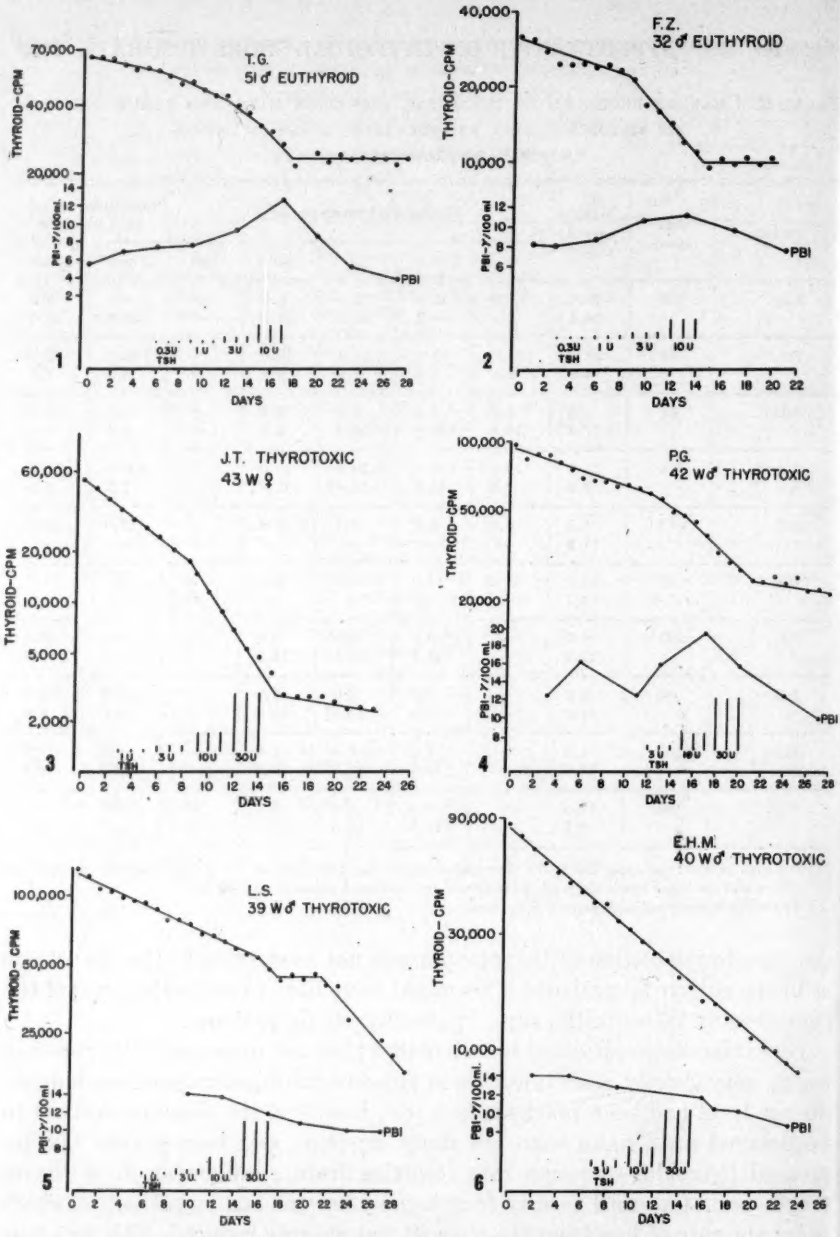

FIG. 1. Typical graded dose-response relationship between increasing doses of thyrotropin and thyroidal secretion rate in a euthyroid patient.

FIG. 2. "All or none" response to thyrotropin in a euthyroid patient.

FIG. 3. "All or none" response to thyrotropin in a thyrotoxic patient.

FIG. 4. Graded dose-response relationship in a thyrotoxic patient.

FIG. 5. Transient inhibition of secretion following cessation of thyrotropin administration in a thyrotoxic patient.

FIG. 6. Failure to obtain a response to as much as 30 units of thyrotropin daily in a thyrotoxic patient.

of the initial rapid secretion rate or an even faster rate was then assumed by the thyroid (Fig. 5). Unfortunately the levels of radioactivity used in these studies did not permit following the patients for more than about one month.

The concentration of stable protein-bound iodine (PBI) in serum underwent the changes which might be expected from the concomitant alterations of thyroidal secretion rate. With faster secretion rates the PBI level rose and with the decreased rate following cessation of thyrotropin administration the level fell. Changes in the concentration of protein-bound I^{131} in general paralleled the changes in concentration of protein-bound stable iodine, but because of the low level of activity in the serum the accuracy of the determinations with I^{131} was considerably less than was possible with stable iodine. In correlation with the rapid exponential loss of iodine from the thyroid in the thyrotoxic patients, the level of serum PBI tended to fall during the course of study. Administration of thyrotropin to some of these patients did not result in elevation of the PBI level, but merely slowed its rate of fall.

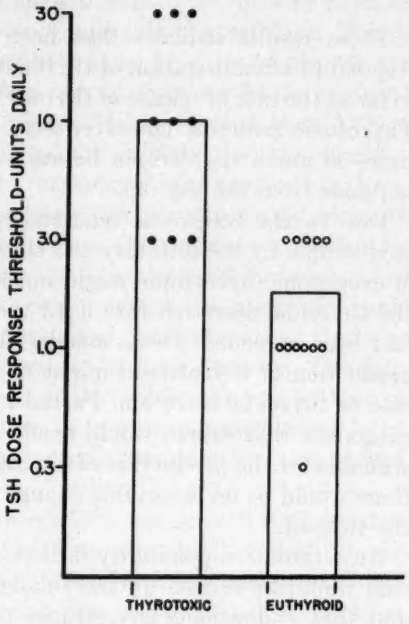

FIG. 7. Comparison of the minimal doses of thyrotropin necessary to elicit a response in euthyroid and in thyrotoxic patients.

There was no correlation between the initial thyroidal secretion rate and the dose-threshold of thyrotropin, but there seemed to be some tendency for patients with faster initial secretion rates to exhibit more rapid maximal rates during administration of thyrotropin.

Although none of the thyrotoxic patients had any clinical evidence of exacerbation of the disease while they were receiving thyrotropin, manifestations of thyrotoxicosis developed in several of the euthyroid patients during this period. Weight loss, tachycardia, palpitation, tremor and increased sweating were sometimes noted. In 2 patients, slight enlargement of the thyroid gland was produced; the enlargement regressed after discontinuation of thyrotropin.

A surprising finding was that in all but 1 of the thyrotoxic patients (Fig. 3) and in 4 of the euthyroid patients (Fig. 2) there seemed to be an "all or none" phenomenon in regard to their response to increasing doses of thyrotropin. Once the threshold dose had been reached, larger doses did not produce a greater rate of discharge from the thyroid. In the other euthyroid patients so studied (Fig. 1), a typical dose-response relationship was observed.

DISCUSSION

These results indicate that both euthyroid and thyrotoxic patients respond to administration of thyrotropin in a qualitatively similar manner so far as the rate of release of thyroid hormone from the gland is concerned. Thyrotoxic patients, however, seem to require approximately five or six times as much thyrotropin before they exhibit an increased discharge of hormone from the thyroid.

Due to the reciprocal relationship existing between the secretion of thyrotropin by the pituitary and thyroxine by the thyroid, added amounts of exogenous thyrotropin might not be expected to produce any change in the thyroidal secretion rate until the endogenous thyrotropin production had been exceeded. Doses smaller than the equivalent daily endogenous production of thyrotropin might merely result in maintaining the initial rate of thyroidal secretion. Partial maintenance of thyroidal secretion by exogenous thyrotropin would result in a rapid, correspondingly decreased stimulus for the production of thyrotropin by the pituitary. Consequently there would be no detectable change in the rate of loss of radioiodine from the thyroid.

An alternative possibility is that an initial increase in thyroidal secretion would be caused by the smaller amounts of exogenous thyrotropin, and that endogenous thyrotropin production would be suppressed as a result of this initial increase in the output of thyroxine. Equilibration would occur at a later interval. Thus thyroidal secretion would first rise and then fall to the initial, pre-thyrotropin level during continuation of the same dose of thyrotropin. Since the latter phenomenon was not observed to occur, the first hypothesis seems more likely. No decline in the response was observed during injection of the smallest effective dosage of thyrotropin over a period of three days. It is therefore plausible that the amount of thyrotropin secreted endogenously would be less than the equivalent of this smallest effective dosage.

It is possible that three days is not an adequate period to allow stabilization to a given level of thyrotropin, but in some patients the same dose was given daily for one week or longer without any diminution of the original response. Unfortunately, when giving thyrotropin at several different dosage levels, it was usually not possible to continue the administration

of any one dosage over a longer period of time and still retain enough radioactivity in the thyroid for accurate measurements by the end of the experimental period.

If one assumes that 1 unit of thyrotropin is roughly equivalent to the amount produced endogenously daily in the euthyroid individual, the failure of reported blood assays to detect consistently an elevated level of thyrotropin in thyrotoxic patients is not surprising. If there is immediate extracellular distribution of 1 unit of thyrotropin throughout 15 liters of extracellular fluid, the level would be .06 milliunit per milliliter. This is below the range of detection in current bioassays. In order to provide the same effect, probably considerably less thyrotropin would be required from a constant endogenous source over a 24-hour period than from a single daily intramuscular injection. Also, it is unlikely that the distribution of thyrotropin would be limited to the extracellular space. It is therefore remarkable that it is possible to detect thyrotropin in the blood of euthyroid individuals at all. Published values (12) indicate a concentration of about 0.3 milliunit per milliliter; this is in the neighborhood of five times as much as would be expected for immediate dilution of 1 unit throughout 15 liters of extracellular fluid, and probably many times as much as might be expected assuming constant 24-hour secretion. The data (12) indicating that the serum PBI level of thyrotoxic patients is three to five times as high as in euthyroid patients is, however, consistent with the difference in threshold response to thyrotropin between the two groups reported in this paper.

The "plateauing" of the secretion rate which was seen in both the hyperthyroid and euthyroid patients following cessation of thyrotropin therapy most likely represents transient inhibition of the endogenous output of thyrotropin. An exactly comparable decrease in thyroidal secretion rate is seen when thyroxine or triiodothyronine is given to euthyroid patients (13). One difference is that inhibition occurs very shortly after administration of thyroxine, whereas it requires twenty-four hours for the stimulation following intramuscular thyrotropin to wear off. This may be related to the route of administration, since Reichlin and Reid (14) found that in rabbits the duration of action of intravenous thyrotropin on the thyroidal secretion rate was much less than that of subcutaneous thyrotropin. The secretion rate decreased as rapidly following an intravenous injection of thyrotropin as it did following administration of thyroxine. A puzzling feature of this inhibition is that in some of our thyrotoxic patients this phenomenon existed without a rise in the serum PBI level having been produced by the previous injection of thyrotropin. Moreover, inhibition of thyroidal secretion was more complete in the euthyroid than in the thyrotoxic subjects.

The data reported here appear to support the hypothesis that Graves' disease is due to an increased secretion of thyrotropin by the pituitary, but they do not provide conclusive proof. It is also possible that the thyrotoxic subjects required a larger dose of thyrotropin before a response was obtained, because of a greater basal level of thyroidal activity unrelated to endogenous thyrotropin secretion. In the absence of more definitive data, it is felt best to leave the nature of the pathogenesis of thyrotoxicosis open to further investigation.

SUMMARY

The effect of various dosages of thyrotropin upon the thyroidal secretion rate has been studied in 20 euthyroid and in 10 thyrotoxic human subjects. The average daily intramuscular dosage level at which an increased secretion rate was first observed was 1.75 U.S.P. units in the euthyroid patients and 10 units in the thyrotoxic patients. In both groups stimulation of thyroidal secretion began well before the twenty-fourth hour following administration of an effective dose of thyrotropin. A period of inhibition began twenty-four hours after the last injection of thyrotropin. This inhibition was transient in 2 of the thyrotoxic patients and in 1 of the euthyroid patients, but persisted for the remainder of the period of study in the other subjects.

Although the data are inconclusive in this respect, they are compatible with the hypothesis that Graves' disease is due to an increased secretion of thyrotropin by the pituitary.

Acknowledgment

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DIFFERENCES IN METABOLISM OF PREDNISOLONE-C^{14} AND CORTISOL-C^{14}

AVERY A. SANDBERG, M.D. AND W. ROY SLAUNWHITE, JR., PH.D.

Roswell Park Memorial Institute, Buffalo, N. Y.

INTRODUCTION

THE effectiveness in various clinical conditions of 1-dehydro-17-hydroxycorticosteroids in much smaller doses than those of cortisol or cortisone is now well established (1, 2). Little is known about the metabolic fate of these new synthetic 1-dehydrosteroids in the human body. The reasons for their greater clinical effectiveness are likewise unclear. In a previous publication it was reported that in man there are certain differences and some similarities between the metabolism of 1-dehydrocortisol and 1-dehydrocortisone (prednisolone and prednisone respectively) and the metabolism of cortisol and cortisone (17-OHCS) (3). In contrast to the latter steroids, a substantial percentage of 1-dehydrosteroid was excreted in the urine as free (unconjugated) and unchanged steroid, following either oral or intravenous administration. Only small amounts of steroids were released by β-glucuronidase hydrolysis of the urine of subjects given 1-dehydrosteroids (Δ^1-17-OHCS). Furthermore, the Δ^1-17-OHCS were not converted to 17-ketosteroids (17-KS) to the same extent as were the 17-OHCS. The administration of large doses (240 mg. per day) of Δ^1-17-OHCS did not interfere with the normal metabolism of C^{14}-cortisol, and the suppression of adrenal function was in all probability mediated through the pituitary gland. The clinical effectiveness of Δ^1-17-OHCS was postulated to be due, at least in part, to the body's inability to metabolize them in the same manner as the 17-OHCS. The plasma concentrations of steroids, determined by the Porter-Silber reaction, were higher following the administration of prednisolone than following administration of cortisol. The rates of clearance of these steroids from the plasma were essentially similar, however. In contrast, Ely, Done and Kelley (4) reported that Δ^1-17-OHCS were cleared from the plasma at a slower rate than cortisol, indicating a longer half-life for the former than for the latter. These authors concluded that the metabolism of 1-dehydrosteroids is slower, and postulated that this might contribute to the greater clinical effectiveness of these compounds.

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In the studies of Slaunwhite and Sandberg (3) and those of Ely, Done and Kelley (4) the concentrations of steroids in the urine and plasma were determined by methods based on the Porter-Silber color reaction (5, 6). This reaction measures only those steroids with a dihydroxyacetone side-chain. It is known that only a portion of the steroids in urine and in plasma can be measured with methods based on the Porter-Silber reaction. Furthermore, 1-dehydrosteroids produce less color in the Porter-Silber reaction than do 17-OHCS (3, 4).

Since almost nothing is known about the metabolites of 1-dehydrosteroids or their chromogenicity in the Porter-Silber reaction, it was felt that more precise methods would be necessary in order to evaluate the metabolism of 1-dehydrosteroids. With this in mind, the synthesis of radioactive prednisolone was undertaken and its metabolism was compared to that of C^{14}-cortisol. This paper deals with the findings of the study.

METHODS AND MATERIALS

Radioactive 4-C^{14}-cortisol (compound F) was secured through the courtesy of the Endocrinology Study Section of the National Institutes of Health. This radioactive cortisol was converted to radioactive 1-dehydrocortisol by a microbiologic procedure (7). Corynebacterium Simplex (A.T.C.C. #6946) was incubated in a medium of nutrient broth with 0.1 per cent yeast extract at pH 7.0 for varying periods of time in the presence of different amounts of nonradioactive cortisol. In general, under these conditions, incubation for longer than twelve hours resulted in loss of 1-dehydrosteroids. Following twenty-four hours of incubation, neither the parent compound nor the 1-dehydrosteroids could be recovered. Incubation for ten to twelve hours resulted in the most efficient conversion of cortisol to prednisolone (Δ^1-F). Therefore, nonradioactive cortisol and 3.2 μc. of 4-C^{14}-cortisol were incubated for twelve hours. The incubation mixture was then extracted immediately with freshly redistilled chloroform. This extract was evaporated to dryness in a vacuum. The residue was dissolved in 0.5 ml. of absolute methanol and 0.5 ml. of water was added. A white precipitate appeared upon cooling. The precipitate was dissolved again in methanol and water and recrystallized. There was no depression of the melting point of these glistening white crystals when they were mixed with authentic prednisolone. Paper chromatography (8) showed only one spot by examination with an ultraviolet scanner and the same localization by radioactivity when the paper strip was passed under a Geiger tube having an ultra-thin window (<120 μg./sq. cm.) (Nuclear-Chicago, C-100 Actigraph). The Rf of the compound was identical with that of standard prednisolone. After the recrystallizations, 52 per cent of the original radioactivity was recovered.

Each patient received an intravenous injection of 0.5 μc. of 4-C^{14}-prednisolone or 0.5 μc. of 4-C^{14}-cortisol. In addition, Patient M.S. received a 50-mg. injection and Subjects G. LaM. and A.K. each a 4-mg. injection of the corresponding nonradioactive steroids as carriers. The steroids were dissolved in 0.5 to 3.0 ml. of ethanol and diluted with saline to a volume of 30 to 50 ml., which was injected intravenously over a period of one to two minutes. All injections were made at 11 A.M. The midpoint of the injection was taken as "zero" time. The methods for collections of blood and urine, for hydrolysis of the various plasma and urinary fractions, for determination of radioactivity in samples, and for the separation of the free steroids following the various hydrolytic procedures into 17-KS

and 17-OHCS by chromatography on Florisil, have been described previously (6, 9). A 2 per cent methanol solution, instead of the 4 per cent solution used by Glenn and Nelson (6), was employed to elute the 17-KS from the Florisil column, since in our hands the 2 per cent solution provided better separation of the 17-KS (including 11-oxygenated 17-KS) from the corticosteroids.

Three patients (2 women and 1 man) initially received an intravenous injection of radioactive prednisolone. After a period of five days, radioactive cortisol was injected. These patients had not been treated with steroids or other hormones in the past. They were in good nutritional condition, had normal liver function, had not lost weight, and had no apparent metastases. Patient M.S. was a 20-year-old woman who had had a small malignant melanoma of the skin removed two weeks before the study. Subjects G. LaM. (35-year-old woman) and A.K. (52-year-old man), with carcinoma of the breast, had undergone simple mastectomies three weeks before injection of the steroids.

RESULTS

All 3 patients excreted over 90 per cent of the injected radioactivity in the urine in a period of forty-eight hours following the injection of either C^{14}-cortisol or C^{14}-prednisolone. The results are shown in Figure 1. The

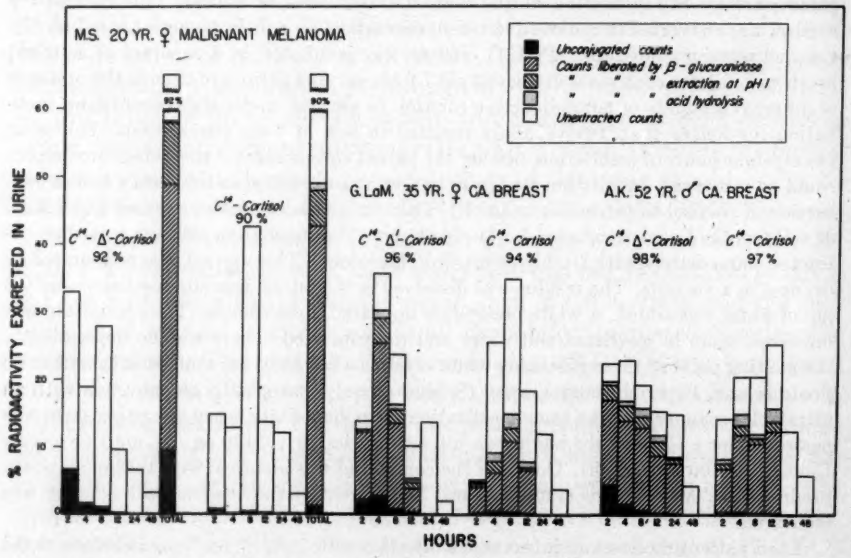

FIG. 1. Radioactivity in the urine of subjects receiving radioactive Δ^1-cortisol and cortisol by intravenous injection. Note substantial amounts of unconjugated metabolites present in the urine following the administration of C^{14}-Δ^1F. The numbers on the abscissa refer to the time (after the injection of the steroid) of completion of the urine collection. The unshaded portions of the individual (excluding the "total") histograms of Subject M.S. do not refer to "unextractable counts"; they merely represent the radioactivity left in the urine following chloroform extraction.

urinary excretion of radioactivity following injection of radioactive prednisolone differed in several respects from that following injection of C^{14}-cortisol. More of the radioactivity was excreted in the urine during the initial eight hours following C^{14}-prednisolone than following C^{14}-cortisol. This phenomenon was especially apparent during the initial four-hour period. In addition, about 10 per cent of the radioactivity following administration of C^{14}-prednisolone appeared in the urine as free unconjugated steroids, whereas less than 2 per cent appeared in the free fraction following administration of C^{14}-cortisol. The largest amount of free 1-dehydrosteroids was excreted in the initial two to four hours, although substantial

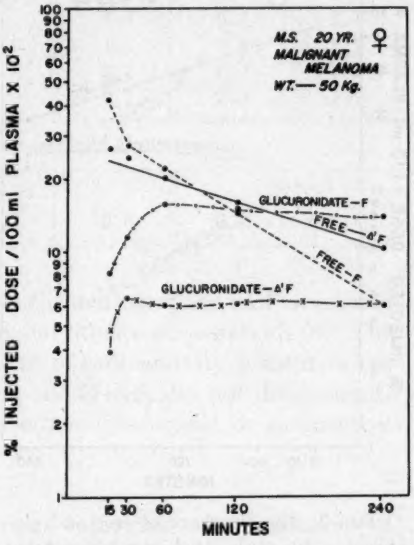

FIG. 2. The clearance of free and glucuronic-acid conjugated steroid metabolites from plasma following the administration of radioactive prednisolone and cortisol intravenously in Subject M. S. She received 50 mg. of carrier of each steroid. In this and the following figures the designations F and Δ^1F do not necessarily imply the presence of these steroids in plasma, but are intended to designate the metabolites following the administration of these steroids.

amounts were present as late as twelve hours following the injection. The amounts of hydrolyzable and ether-extractable radioactive metabolites excreted primarily as glucuronidates and sulfates were significantly larger (especially in Patients G. LaM. and A.K.) following the injection of prednisolone than following the injection of cortisol.

Paper chromatography (8) of a portion of the combined urinary free fractions after C^{14}-prednisolone revealed that the preponderant part of the radioactivity was located in an area with an Rf corresponding to that of standard prednisolone. In addition, this area of radioactivity was absorbed in ultraviolet, and reduced triphenyl-tetrazolium chloride—findings which indicated the presence of a $\Delta^{1,4}$-3-ketosteroid and an α-ketol side-chain, respectively. Three other areas with small amounts of radioactivity

and Rf's greater than those of prednisolone were present also. The identification of these and some of other metabolites of Δ^1-17-OHCS has not been possible, due to lack of standard reference compounds. The small amount of radioactivity present in the free fraction of urine following C^{14}-cortisol administration was insufficient for definite characterization of the metabolites by paper chromatography.

In Figures 2–4 are shown the levels of free steroids and the glucuronic-acid conjugated steroid metabolites in the plasma following the injection

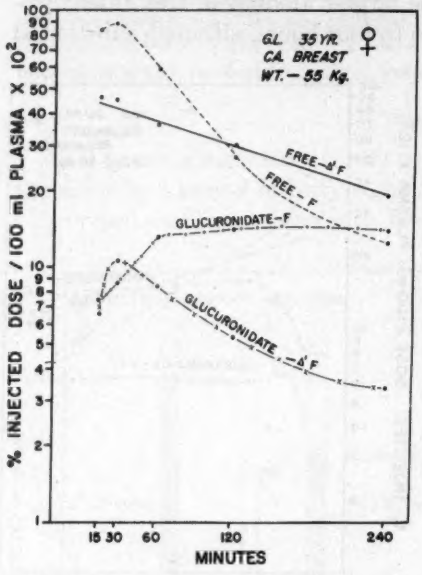

FIG. 3. The clearance of free and glucuronic-acid conjugated steroid metabolites from plasma following the intravenous administration of C^{14} prednisolone and cortisol, in Subject G. LaM.

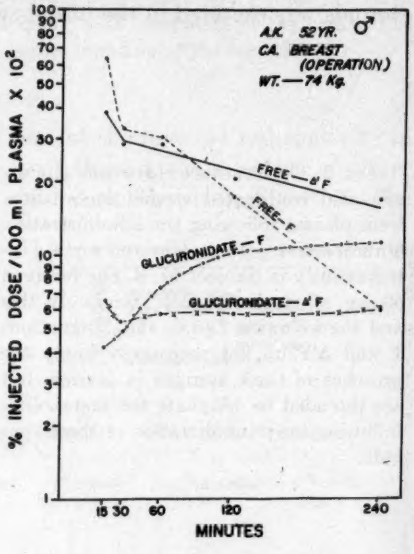

FIG. 4. The clearance of free and glucuronic-acid conjugated steroid metabolites from plasma following the intravenous administration of C^{14} prednisolone and cortisol, in Subject A. R.

of C^{14}-prednisolone or C^{14}-cortisol. During the initial sixty minutes the concentration of free steroid radioactivity was lower following injection of C^{14}-prednisolone than following injection of C^{14}-cortisol. After that period the levels were higher for C^{14}-prednisolone. The glucuronidate levels were higher following C^{14}-cortisol, although the peak of glucuronidate radioactivity was reached earlier following C^{14}-prednisolone.

The steroid conjugates released by hydrolysis of plasma for forty-eight hours at pH 1 (presumed to be primarily sulfates) and those released by hydrolysis with strong acid and heat indicated that there was no substantial variation between the two steroids (Table 1).

TABLE 1. RADIOACTIVITY IN STEROID CONJUGATES RELEASED BY HYDROLYSIS OF PLASMA FOLLOWING INTRAVENOUS INJECTION OF RADIOACTIVE PREDNISOLONE (C^{14}-Δ^1F) OR RADIOACTIVE CORTISOL (C^{14}-F)

Minutes after injection	Per cent of dose/100 ml. of plasma $\times 10^2$					
	Patient M.S.		Patient G.LaM.		Patient A.K.	
	Δ^1-F	F	Δ^1-F	F	Δ^1-F	F
Hydrolysis for 48 hrs. at pH 1 (sulfates?)						
15	0.8	1.3	1.4	0.9	3.4	1.2
30	5.3	18.9	1.4	1.4	0.8	0.4
60	1.4	2.6	1.6	1.5	0.8	1.7
120	1.4	3.4	2.4	2.1	0.9	1.7
240	0.9	2.0	1.9	2.7	2.5	1.1
Hydrolysis with strong acid and heat						
Combined specimens	0.8	0.4	0.9	0.6	0.7	1.1

Part of each total urinary extract was chromatographed on Florisil and the 17-KS separated from the 17-OHCS and other corticosteroids (6). The results are shown in Table 2. The amount of radioactivity present in the 2 per cent methanol fraction, containing the 17-KS, did not differ significantly following the administration of either C^{14}-cortisol or radioactive prednisolone.

TABLE 2. RADIOACTIVITY IN VARIOUS FRACTIONS FOLLOWING CHROMATOGRAPHY OF URINARY EXTRACTS ON FLORISIL*

Patient	2% Methanol (17-KS)		25% Methanol (free steroids)		25% Methanol (conjugated steroids)	
	F	Δ^1-F	F	Δ^1-F	F	Δ^1-F
M.S.	6.0	7.7	4.2	34.8	89.8	57.5
G.LaM.	10.4	7.1	3.6	16.8	86.0	76.1
A.K.	5.7	8.4	2.0	16.3	92.3	75.3
Mean	*7.4*	*7.7*	*3.3*	*22.6*	*89.4*	*69.6*

* In each case all urinary extracts were combined (keeping the free and previously conjugated steroids separate) and chromatographed on Florisil. The results are shown as the percentage of the total counts removed from the chromatographic column. The 2%-methanol fraction contains the 17-KS and probably other steroids.

DISCUSSION

From the data of the present paper and those of previous publications (3, 4) some of the possible mechanisms can be postulated for the greater metabolic effectiveness of 1-dehydrosteroids, compared to cortisol and cortisone. The use of radioactive prednisolone and cortisol has made such an appraisal more reliable. Previous studies with large doses of steroids depended on methods based upon the Porter-Silber reaction (3, 4). This reaction measures only those steroids with a dihydroxyacetone side-chain. Hence, only about a third of administered steroids can be accounted for in the urine with the Porter-Silber reaction. On the other hand, following the administration of radioactive prednisolone and cortisol, over 90 per cent of the radioactivity of each steroid can be accounted for in the urine in forty-eight hours.

In the previous studies with large doses of nonradioactive steroids (3) administered orally or intravenously, it was shown that following the administration of Δ^1-17-OHCS the ratio of conjugated metabolites (glucuronidates) to free steroid metabolites in the urine (determined by the Porter-Silber reaction) was usually less than 1.0 and sometimes as low as 0.05. Conversely, following the administration of 17-OHCS, the ratio was about 10 and as high as 30. Since the recoveries of Porter-Silber reacting steroids in both cases were similar (20–30 per cent), it is apparent that the amounts of these steroid metabolites conjugated with glucuronic acid and excreted following the administration of Δ^1-17-OHCS were much smaller than those following administration of 17-OHCS. The studies with C^{14}-labelled prednisolone and cortisol reveal that following their administration the preponderant amount of urinary steroid metabolites is excreted in a form conjugated with glucuronic acid (at least 40 per cent of the administered dose). This would seem to indicate that most of the Δ^1-17-OHCS metabolites and a substantial part of the metabolites of 17-OHCS do not retain the dihydroxyacetone side-chain and, hence, cannot give rise to the Porter-Silber reaction. This is consonant with the recent demonstration that a considerable portion of administered cortisol appears in the urine as glycols (10). In addition, the hydrolyzable conjugated steroid metabolites of C^{14}-prednisolone appear in the urine in somewhat larger amounts and more rapidly than those of C^{14}-cortisol. It would seem that once the Δ^1-17-OHCS are metabolized and conjugated, they are excreted somewhat faster than the metabolites of 17-OHCS.

Of great interest was the excretion of substantial amounts (10 to 15 per cent) of free Δ^1-17-OHCS in the urine following the administration of small (radioactive) or large doses (3) of these steroids, as compared to the negligible amounts (<2 per cent) of free steroids observed following the administration of 17-OHCS. The free steroids in the former case consisted

mainly of unmetabolized Δ^1-17-OHCS, whereas the free steroids following cortisol or cortisone administration consist primarily of metabolites of these steroids (11, 12). Apparently the body is unable to metabolize Δ^1-17-OHCS in the same manner as it metabolizes cortisol or cortisone. Several studies published since the termination of this work are of interest. Gray *et al.* (13) reported that, following the oral administration of prednisone or prednisolone, the two compounds present in largest amount in the urine were prednisone or prednisolone; in smaller amounts were the 20-alcohol derivatives of the two steroids. Vermeulen (14) identified prednisone, prednisolone and the 20β-alcohol derivative of prednisone in the urine of 2 patients with hypopituitarism treated with oral prednisone. No tetrahydro derivatives were found. The findings of Gray *et al.* and of Vermeulen definitely point towards the difficulty encountered by the human body in the reduction of ring A of $\Delta^{1,4}$-3-ketosteroids and the presence of large amounts of unmetabolized steroid in the urine. *In vitro* studies by Tomkins (15) indicate that although the enzyme systems of the rat liver reduce the Δ^4-3-ketone group in cortisol, they are ineffective in reducing the similar group in prednisolone.

Further evidence that the human subject does not metabolize Δ^1-17-OHCS in the same manner as 17-OHCS can be obtained from the clearance of free radioactive steroids from the plasma. Before this subject can be discussed, the following observations need clarification. One or all of several factors may account for the fact that the levels of radioactivity in the free fraction of plasma are lower during the initial sixty minutes following the injection of radioactive prednisolone than they are following the injection of C^{14}-cortisol: 1) excretion of a significant amount of free C^{14}-prednisolone in the urine, especially during the initial fifteen minutes, 2) a much larger metabolic pool for C^{14}-prednisolone than for C^{14}-17-OHCS, and 3) faster equilibration of C^{14}-prednisolone in a body pool.

As has been discussed in previous publications (16, 17), the most probable explanation for the steeper fall in the radioactivity of the free steroid fraction during the initial thirty minutes, especially following injection of C^{14}-cortisol, is that this period represents the time required for the steroid molecules to come into equilibrium in the body compartments. Since, following that initial period, the logarithms of the values of free steroid radioactivity fall approximately in a straight line, it would appear that during the period from one to four hours equilibrium exists between the free steroid pool and the plasma. Based on this assumption it is possible to calculate the half-life of the free steroids in plasma. Examination of Figures 2–4 reveals that metabolism of the free steroids of C^{14}-prednisolone is slower than that of the free steroids of C^{14}-cortisol. The half-life of the latter (based on the data of the present study as well as of studies published

previously) is approximately 80–100 minutes (16, 17), whereas that of C^{14}-prednisolone (calculated from Figures 2–4) is approximately 200 to 220 minutes. These findings are in accord with those of Ely, Done and Kelley (4). These authors determined the clearance of Porter-Silber chromogens from the plasma following the administration of large doses of either hydrocortisone or prednisolone, and estimated that the mean half-life of prednisolone was 192 minutes compared to 90 minutes for cortisol.

If the metabolites of prednisolone are excreted in the urine more rapidly than those of 17-OHCS (as judged by the excretion of administered radioactivity), how can one explain the effectiveness of prednisolone on the basis of slower metabolism? It is now well substantiated that the corticosteroids are metabolically effective only while still unconjugated (free) and having a Δ^4-3-keto configuration in ring A. The first steps in the metabolism of these steroids involve the formation of the dihydro and tetrahydro forms of the corticosteroids (reduction of the double bond and 3-ketone, respectively), with resultant loss of biologic activity. Furthermore, following reduction of the Δ^4-3-keto part of the steroid molecule, conjugation occurs, primarily with glucuronic acid. These conjugates have no metabolic effects. Thus, the rate of clearance of unconjugated steroid should be an indication of the metabolism of the "biologically active" steroid in the body. In the case of cortisol, however, the concentration of unconjugated steroid is not a true measure of the concentration of "biologically active" steroid. About 50 per cent of unconjugated steroid in plasma following administration of cortisol consists of compounds already reduced in ring A and they are already metabolically inert (16). On the other hand, in the case of prednisolone, it is probable that most of the unconjugated steroid in the plasma consists of unmetabolized compound. This statement gains support not only from the data showing substantial amounts of free and unchanged Δ^1-steroid excreted in the urine, but also from the finding that as late as twelve hours following the injection of C^{14}-prednisolone a significant amount of free prednisolone is being excreted, indicating its continued presence in plasma at that time. It is possible then, that at least one cause of the greater effectiveness of Δ^1-steroids compared with cortisol is the slower metabolism of these steroids in the body and the higher concentration of the "biologically active" fraction.

However, the slower metabolism and the slower plasma clearance of Δ^1-steroids may not be the only, or even the major factor in their clinical effectiveness. Since little is known about the mode of action of steroids in the body, it is possible that the differences in the biologic properties and potencies of steroids may not be related solely to differences in rates of excretion and catabolic degradation. There are many other possible explanations for the clinical effectiveness of the Δ^1-steroids other than those suggested by the data of the present study.

Although the levels of glucuronic-acid conjugated steroid metabolites in the plasma following administration of C^{14}-prednisolone are lower than those following administration of C^{14}-cortisol, the difference may not be due primarily to failure of conjugation. These lower levels are probably related to faster clearance of the Δ^1-steroid glucuronidates from the plasma, as indicated by their rapid excretion in the urine.

In a previous study with nonradioactive steroids it was demonstrated that the conversion of 1-dehydrosteroids to 17-KS does not take place to the same extent as does the conversion to 17-OHCS; in fact there is a decrease in urinary 17-KS during the administration of 1-dehydrosteroids (3). In the present study the 2 per cent methanol fraction containing the 17-KS (obtained from Florisil chromatography) exhibited as much radioactivity following injection of radioactive prednisolone as following injection of radioactive cortisol. Possible explanations of this discrepancy are: 1) the 17-KS originating from 1-dehydrosteroids are much less chromogenic in the Zimmermann reaction, or 2) the 2 per cent methanol fraction contains steroids other than 17-KS.

SUMMARY

1. The metabolism of C^{14}-prednisolone has been compared to that of C^{14}-cortisol in 3 human subjects, 1 with malignant melanoma and 2 with carcinoma of the breast.

2. Following intravenous administration of C^{14}-prednisolone, the urinary excretion of radioactivity was slightly more rapid than that following C^{14}-cortisol. More than 90 per cent of the dose of radioactivity appeared in the urine in the forty-eight hours following the injection of either steroid.

3. About 10 per cent of C^{14}-prednisolone appeared in the urine as free, unchanged steroid. This is at least five times the amount of free steroid present following administration of C^{14}-cortisol.

4. The more pronounced clinical effectiveness of the Δ^1-17-hydroxycorticosteroid, prednisolone, when compared to cortisol is attributed to the slower metabolism of the unconjugated unreduced "biologically active" steroid. This finding would indicate an inability of the body to metabolize Δ^1-17-hydroxycorticosteroids in the same manner or at the same rates as 17-hydroxycorticosteroids.

Acknowledgments

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DEHYDROEPIANDROSTERONE AND ANDROSTERONE LEVELS IN HUMAN PLASMA. EFFECT OF AGE AND SEX; DAY-TO-DAY AND DIURNAL VARIATIONS*

CLAUDE J. MIGEON, M.D., ANNE R. KELLER, B.A., BETTY LAWRENCE AND THOMAS H. SHEPARD, II, M.D.†

The Department of Pediatrics, The Johns Hopkins University School of Medicine, and the Harriet Lane Home of The Johns Hopkins Hospital, Baltimore, Maryland, and the Department of Biological Chemistry, University of Utah College of Medicine, Salt Lake City, Utah

METHODS have been described for the measurement of total 17-ketosteroids (17-KS) occurring in human plasma (1) and for their fractionation (2). Dehydroepiandrosterone (DHA) and androsterone have been isolated and found to be the principal 17-KS in the peripheral circulation of normal man (3, 4). The present study was carried out in order to obtain further information on the levels of DHA and androsterone in normal subjects and to ascertain the effect of age and sex on the concentrations of these steroids. It was also thought to be of interest to determine the diurnal variation as well as the day-to-day changes of the 17-KS levels in the same subjects.

MATERIALS AND METHODS

A 40-ml. sample of blood was drawn from each experimental subject between 8:30 and 9:30 A.M., except in the study of diurnal variations, when blood was obtained at various times of the day. The blood samples were heparinized. They were centrifuged within forty-five minutes following collection and the plasma was immediately separated from the red cells. When the determination was not carried out immediately, the plasma was kept in a freezer.

Dehydroepiandrosterone (DHA) and androsterone levels in plasma were determined by the technique described by Migeon and Plager (2), consisting of the following steps: ethanol precipitation, 48-hour continuous extraction with ether at pH 0.8, washing with $NaHCO_3$, chromatography on a Florisil column, removal of the phenols with 1*N* NaOH, paper chromatography with the light-petroleum:methanol:water (100:85:15) system of

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† Present address: Department of Pediatrics, Medical School, University of Washington, Seattle, Washington.

TABLE 1. MEAN LEVELS OF 17-KS IN THE PLASMA OF 65 NORMAL ADULT MALES AT DIFFERENT AGES

Age group	No. of subjects	Plasma DHA (μg./100 ml.) (mean ± S.D.)*	Plasma androsterone (μg./100 ml.) (mean ± S.D.)
20–29 yrs.	24	48.2 ± 15.1	25.0 ± 10.7
30–39 yrs.	14	44.8 ± 20.2	21.6 ± 10.5
40–49 yrs.	10	28.1 ± 12.4	19.8 ± 8.1
50–59 yrs.	7	20.5 ± 8.2	11.6 ± 10.0
60–69 yrs.	5	10.3 ± 10.0	2.2 ± 3.0
70–79 yrs.	3	3.9	2.3
80–89 yrs.	2	2.0	0

* Standard Deviation.

Bush (5), elution from the paper, and performance of the micro-Zimmermann reaction (6) with the eluate.

The influence of age on the levels of DHA and androsterone in normal adult males was studied in 65 subjects (Nos. 1–65) ranging in age from 20 to 87 years (Table 1). When several observations were made on the same subject, the first value only was retained for the present study. The variation of the levels from day to day was determined in 5 of these male subjects (Nos. 9, 11, 19, 20 and 28) (Table 2). The diurnal variation was studied in 10 other men of this group, as well as in 3 normal females (Subjects 66–68) (Table 3).

The effect of the menstrual cycle on the levels of DHA and androsterone had been studied previously in 8 normal females (Subjects 69–76) ranging in age from 21 to 29 years (7). Blood had been collected two days, two weeks and three weeks after the beginning of menstruation, and two days after the beginning of the following period. Because of a possible variation in the middle of the cycle, the same study was carried out on 8 additional females (Subjects 77–84) (Table 4). The concentration of 17-KS in the plasma of 12 normal postmenopausal women, 46 to 95 years of age, was also determined (Subjects 85–96) (Table 5).

Finally, the steroid levels were studied in 8 well babies under 1 year of age (Subjects

TABLE 2. DAY-TO-DAY VARIATION OF PLASMA 17-KS LEVELS IN 5 NORMAL ADULT MALES

Subjects			Plasma DHA (μg./100 ml.)			Plasma androsterone (μg./100 ml.)		
No. & Name		Sex & Age	Sample 1	Sample 2	Sample 3	Sample 1	Sample 2	Sample 3
#9	P.H.	M 24	48.5	44.3	51.0	14.5	27.0	31.0
#11	C.P.	M 25	47.0	43.0	32.5	20.3	9.0	13.5
#19	P.B.	M 26	69.0	60.5	61.0	26.0	24.5	24.0
#20	M.R.	M 26	43.0	60.0	48.0	33.8	37.0	32.0
#28	M.H.	M 31	82.5	94.0	87.0	24.5	16.2	18.0

TABLE 3. DIURNAL VARIATION OF PLASMA 17-KS LEVELS IN 13 NORMAL ADULT SUBJECTS (10 MEN AND 3 WOMEN)

Subjects		Plasma DHA (μg./100 ml.)										Plasma androsterone (μg./100 ml.)									
No. & Name	Sex & Age	8 A.M.	9 A.M.	10 A.M.	12 NOON	4 P.M.	10 P.M.	2 A.M.	4 A.M.	6 A.M.	8 A.M.	8 A.M.	9 A.M.	10 A.M.	12 NOON	4 P.M.	10 P.M.	2 A.M.	4 A.M.	6 A.M.	8 A.M.
#47 P.	M 48	31.8					13.9	23.4	19.2		27.8	20.3					18.8	13.5	13.5		20.4
#19 P.B.	M 26	69.0					53.5	56.0	57.0		70.0	26.0					15.5	13.0	19.3		23.8
#66 C.C.	F 29	31.2					26.4	21.0	9.6	33.6	30.6	19.0					16.8	12.0	7.4	21.3	18.6
#39 H.B.	M 40	38.5					30.1		28.0	43.7	32.5	30.0					19.6	25.0	22.5	33.0	30.4
#40 B.R.	M 40	36.0					29.4	31.9	31.9		38.4	23.0					19.3	12.2	19.8		20.7
#52 F	M 54	55.0	54.5	36.2	28.8	35.5					46.5	27.6	20.4	16.3	14.5	8.0					23.5
#38 L.	M 39	22.0	17.5	14.5	13.5	11.5					22.3	16.5	16.5	16.0	11.0	11.0					18.2
#49 D.	M 51	15.2		5.0	10.4	10.0					21.0	7.5	8.2	5.0	7.1	6.0					9.0
#44 B.E.	M 45	31.2	29.5	23.2	25.5	26.0						17.5	12.9	10.1	9.5	13.3					
#3 F.F.	M 22	36.5			30.5	28.2	25.8	24.0	29.2		33.3	23.8			23.4	22.0	20.4	17.4	20.5		25.1
#67 S.B.	F 23	65.5			60.0	72.0	65.5	33.8	65.0	61.0		21.0			23.0	32.0	29.5	22.7	16.6	28.5	31.5
#68 P.C.	F 25	36.2			32.2	24.2	20.6	14.5	36.0		34.0	13.0			10.3	11.2	10.0	8.9	13.1		16.0
#41 M.T.	M 41	23.7			28.5		26.0	35.0	24.2	27.5	31.0	10.7			13.2	8.9	8.5	3.5	4.5	5.0	14.2

TABLE 4. EFFECT OF THE MENSTRUAL CYCLE ON THE LEVELS OF PLASMA 17-KS IN 16 NORMAL ADULT FEMALES

Subjects		Plasma 17-KS (μg./100 ml.)							
		DHA				Androsterone			
No. and Name	Age (yrs.)	1*	2	3	4	1*	2	3	4
69 J.D.	25	45.5	56.5	33.0	—	29.5	36.5	17.0	—
70 P.W.	27	34.0	50.0	32.5	39.0	21.0	30.0	19.5	21.4
71 E.K.	24	38.0	50.0	29.5	—	25.5	25.6	9.7	—
72 A.S.	27	51.0	68.0	39.5	—	32.5	27.0	28.5	—
73 T.D.	29	19.8	18.2	14.3	16.4	10.0	3.3	10.1	11.5
74 L.H.	25	45.0	58.5	54.0	45.0	19.1	15.6	13.9	16.3
75 M.F.	21	58.0	81.0	63.5	51.0	13.5	17.5	12.0	15.3
76 R.C.	26	59.5	50.0	48.0	—	37.0	26.2	23.0	—
77 S.R.	22	9.1	14.0	8.1	19.7	0	7.4	0	4.7
78 G.S.	24	34.0	32.6	22.0	36.5	48.5	38.0	22.5	40.0
79 L.F.	25	50.5	54.0	36.0	45.4	9.0	13.5	26.5	23.8
80 C.W.	23	52.0	32.6	—	46.0	24.5	19.3	17.9	37.8
81 A.B.	23	41.6	26.0	39.5	—	24.5	17.5	26.0	—
82 J.D.	24	78.0	53.0	68.0	—	25.0	32.5	22.6	—
83 P.B.	22	16.6	16.6	17.0	0	1.7	0	0	0
84 J.F.	25	64.0	—	50.4	—	25.0	38.0	21.6	—
Average ± S.D.		43.5 ±17.5	44.1 ±19.0	37.0 ±16.9	33.2 ±16.4	21.5 ±12.3	21.7 ± 8.2	17.0 ± 8.3	18.9 ±13.1

* 1 = 2 days after beginning of menstruation.
2 = 2 weeks after beginning of menstruation.
3 = 3 weeks after beginning of menstruation.
4 = 2 days after beginning of following menstruation.

97–104), in 9 normal children between 1 and 9 years (Subjects 105–113) and in 11 normal children between 10 and 16 years of age (Subjects 114–124). In 4 of the babies, plasma collected at intervals of five to seven days was pooled.

RESULTS

Effect of age on plasma 17-KS levels in normal adult males

Table 1 and Figure 1 show that the levels of DHA and androsterone were at their maximum between 20 and 39 years of age. In the few subjects studied who were 70 years of age or over, no significant amount of 17-KS could be detected in the plasma. There is also some suggestion that the levels of DHA decreased faster than those of androsterone during the fourth and the fifth decades of life.

It will be noted from Figure 1 and from the high standard deviations of

TABLE 5. LEVELS OF 17-KS IN THE PLASMA OF 12 POSTMENOPAUSAL WOMEN

Subject		Plasma 17-KS (μg./100 ml.)	
No. and Name	Age (yrs.)	DHA	Androsterone
85 M.G.	46	18.0	8.6
86 D.G.	52	12.5	12.0
87 D.	55	2.0	0
88 S.E.	58	0	0
89 E.E.	59	0	8.0
90 I.R.	70	0	0
91 E.N.	70	7.5	0
92 B.R.	75	0	0
93 B.M.	76	0	0
94 M.L.	78	0	0
95 M.N.	93	0	0
96 D.N.	95	0	0

the mean recorded in Table 1, that there were large individual variations within each age group.

Day-to-day changes in plasma 17-KS levels in normal adult males

Blood samples were obtained from 5 men at approximately three-week intervals and the plasma concentrations of DHA and androsterone were determined. As shown in Table 2, there was some variation of the levels in each individual. The variation at specific hours during two consecutive days is shown in Figure 2 and Table 3; it was not essentially different from that found at three-week intervals.

Diurnal changes in plasma 17-KS levels in normal adult subjects

The variations in the plasma levels of DHA and androsterone during a 24-hour period are shown in Table 3. Great differences in the values for the 8 A.M. control samples were found in this group of subjects. This could be attributed to the fact that these individuals were of different ages; but, as shown in the preceding paragraph, it must be kept in mind that in a single age group large individual variations can occur. It was not possible, therefore, to apply a statistical test to the average levels obtained at different times of the day. For this reason, the various DHA values obtained in one subject were compared to his 8 A.M. control value, and plotted according to the method shown in Figure 2. The mean of the changes observed at a given time of the day for the entire group of subjects was calculated. It was then compared to the mean of the changes found in the second 8 A.M. values, and the level of significance of the difference was determined

TABLE 4. EFFECT OF THE MENSTRUAL CYCLE ON THE LEVELS OF PLASMA 17-KS IN 16 NORMAL ADULT FEMALES

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70 P.W.	27	34.0	50.0	32.5	39.0	21.0	30.0	19.5	21.4
71 E.K.	24	38.0	50.0	29.5	—	25.5	25.6	9.7	—
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73 T.D.	29	19.8	18.2	14.3	16.4	10.0	3.3	10.1	11.5
74 L.H.	25	45.0	58.5	54.0	45.0	19.1	15.6	13.9	16.3
75 M.F.	21	58.0	81.0	63.5	51.0	13.5	17.5	12.0	15.3
76 R.C.	26	59.5	50.0	48.0	—	37.0	26.2	23.0	—
77 S.R.	22	9.1	14.0	8.1	19.7	0	7.4	0	4.7
78 G.S.	24	34.0	32.6	22.0	36.5	48.5	38.0	22.5	40.0
79 L.F.	25	50.5	54.0	36.0	45.4	9.0	13.5	26.5	23.8
80 C.W.	23	52.0	32.6	—	46.0	24.5	19.3	17.9	37.8
81 A.B.	23	41.6	26.0	39.5	—	24.5	17.5	26.0	—
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84 J.F.	25	64.0	—	50.4	—	25.0	38.0	21.6	—
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91 E.N.	70	7.5	0
92 B.R.	75	0	0
93 B.M.	76	0	0
94 M.L.	78	0	0
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96 D.N.	95	0	0

the mean recorded in Table 1, that there were large individual variations within each age group.

Day-to-day changes in plasma 17-KS levels in normal adult males

Blood samples were obtained from 5 men at approximately three-week intervals and the plasma concentrations of DHA and androsterone were determined. As shown in Table 2, there was some variation of the levels in each individual. The variation at specific hours during two consecutive days is shown in Figure 2 and Table 3; it was not essentially different from that found at three-week intervals.

Diurnal changes in plasma 17-KS levels in normal adult subjects

The variations in the plasma levels of DHA and androsterone during a 24-hour period are shown in Table 3. Great differences in the values for the 8 A.M. control samples were found in this group of subjects. This could be attributed to the fact that these individuals were of different ages; but, as shown in the preceding paragraph, it must be kept in mind that in a single age group large individual variations can occur. It was not possible, therefore, to apply a statistical test to the average levels obtained at different times of the day. For this reason, the various DHA values obtained in one subject were compared to his 8 A.M. control value, and plotted according to the method shown in Figure 2. The mean of the changes observed at a given time of the day for the entire group of subjects was calculated. It was then compared to the mean of the changes found in the second 8 A.M. values, and the level of significance of the difference was determined

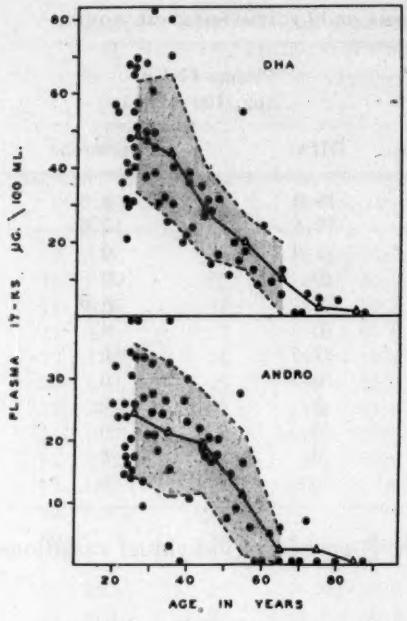

FIG. 1. Plasma levels of DHA and androsterone in normal males. The black circles represent individual values. The open triangles are the mean values at the various decades of age, and the shaded area shows the value of one standard deviation from the mean.

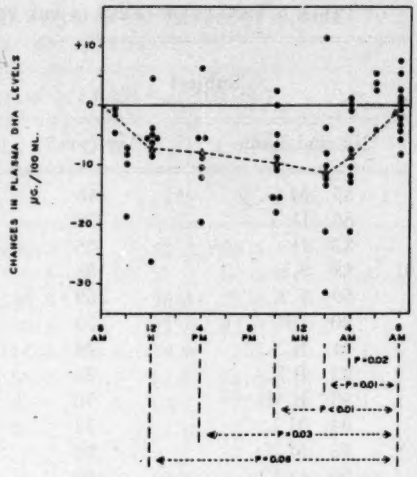

FIG. 2. Diurnal variation of plasma levels of DHA in normal adults. The various values are compared to the first 8 A.M. levels, and the changes (in μg. per 100 ml. of plasma) are plotted against the time of the day. The black circles show the individual changes and the open triangles, their mean values. The significance of these changes was tested against the variation obtained on the second 8 A.M. levels; the p values are indicated at the lower part of the figure.

by the "student" t test (8). The results tested were significantly lower than the 8 A.M. values. However, the decrease observed at noon was not significantly different from that observed at either 4 P.M., 10 P.M., 2 A.M. or 4 A.M.

Effect of the menstrual cycle on plasma 17-KS levels in normal adult females

The mean values for the concentrations of DHA and of androsterone in the plasma of 16 normal females ranging in age from 21 to 29 years are shown in Table 4. It may be seen that there were large individual variations. The average was slightly, but not significantly, lower than that found in normal males of the same age group. There was no statistically significant difference in the levels observed two days, two weeks and three weeks after the onset of menstruation.

In 12 postmenopausal women (Subjects 85–96) ranging in age from 46 to 95 years, the plasma 17-KS levels were low or zero (Table 5)..

Plasma 17-KS levels in children

The values obtained in 22 infants and children are shown in Figure 3. These levels are compared with those found at birth in umbilical cord blood (9) and with those found in normal males, 20–29 years of age. Following birth, the concentrations of 17-KS fell rapidly to zero and stayed at this level until the age of 5–7 years. From 8 years of age on, the values rose progressively to adult levels.

DISCUSSION

Under the influence of the tropic hormones of the pituitary gland, the adrenals, testes and perhaps ovaries secrete androgens (Fig. 4).

Three steroids with 19 carbons have been isolated from beef adrenals, namely, Δ^4-androstene-3,11,17-trione (11), androstane-3β, 11β-diol-17-one (12), and Δ^4-androstene-3,17-dione (13). The latter compound was tentatively identified in extracts of fetal human adrenals along with Δ^4-androstene-11β-ol-3,17-dione and DHA (14). Extracts of adrenal-vein blood

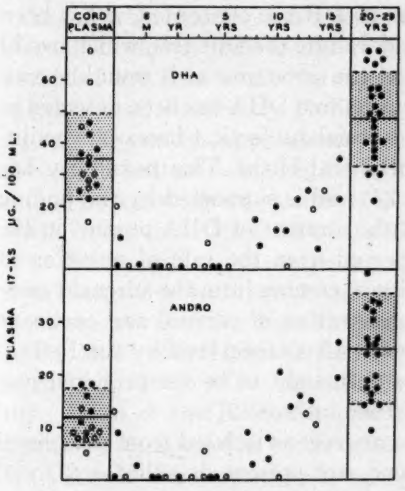

FIG. 3. Plasma levels of DHA and androsterone during childhood and adolescence. The open circles are the value obtained in females and the black circles are those obtained in males. These values are compared with those found in umbilical cord blood and in the blood of normal adult males, the full line being the average level and the dotted line showing the value of one standard deviation.

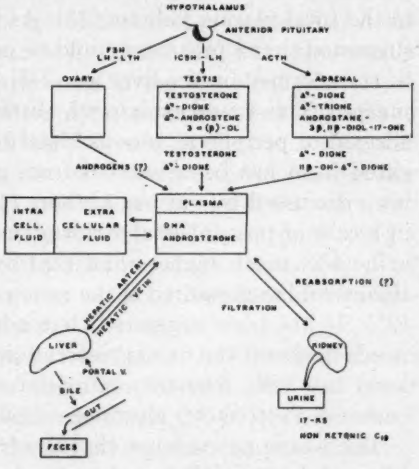

FIG. 4. Present status of our knowledge of androgen metabolism in mammals. The steroids present in peripheral plasma are not necessarily the representation of the products of glandular secretion. Their chemical form and concentration are the overall result of several factors—production by the endocrine glands, distribution in the various body compartments, catabolism by the liver, and rate of excretion by the kidneys.

from cats, rats and sheep were found to contain some Δ^4-androstene-11β-ol-3,17-dione (15) and this steroid was also detected in the adrenal-vein blood of man (16).

Testosterone has been isolated from perfusates of human testis (17) as well as from the testes of other mammals (18, 19, 20). It was also found in spermatic-vein blood of dogs, along with Δ^4-androstene-3,17-dione (21).

No C_{19} steroids have been isolated from ovaries up to the present, but there is evidence that these organs can produce such compounds (22).

Since the foregoing androgens are excreted in the general circulation, one would expect they would be present also in free form in the peripheral blood. However, very small amounts of α-β unsaturated ketones giving rise to the Zimmermann reaction, and minimum amounts of free DHA, have been detected in the extracts of untreated plasma (10). It is possible that these steroids are metabolized so rapidly that they cannot be easily detected in peripheral blood by our present methods. Certain facts would tend to support this theory, since fifteen minutes after the intravenous administration of 4-C^{14}-testosterone only 8–10 per cent of the dose remains in the total plasma volume (23). As far as DHA is concerned, it has been suggested that a precursor could be produced by the adrenals, which would be transformed by the liver into DHA at the same time as it would be conjugated. This would explain why little or no free DHA has been detected in adrenal or peripheral venous blood of normal subjects, whereas its conjugated form has been isolated from peripheral blood. This possibility has been discussed by various authors (4, 24) and is supported by the finding in a case of postpubertal virilism that the amount of DHA present in the urine was much higher than that expected from the rate of secretion of this steroid as measured in the venous blood coming from the adrenal tumor (25). It has been suggested that administration of cortisol and cortisone would increase the urinary excretion of DHA as measured by the Pettenkofer test (26), whereas corticosterone is thought to be the principal precursor of Pettenkofer chromogens by other authors (27).

There is some evidence that the two androgens isolated from peripheral human plasma, DHA and androsterone, are present as sulfates (2) and only very small amounts of androsterone and etiocholanolone have been detected following β-glucuronidase hydrolysis (10). In order to reconcile the fact that androgens conjugated with sulfuric acid appear to be present in blood in the greatest concentration, whereas urinary 17-ketosteroids are mainly conjugated with glucuronic acid, we would have to postulate that the rate of excretion of glucuronosides is more rapid than that of sulfates.

It is difficult to assess the biologic importance of dehydroepiandrosterone and androsterone in peripheral human plasma. Although it must be stressed again that they are present in a conjugated form and therefore

they probably represent the elimination products of some more active hormones, the physiologic variations in their levels is an interesting phenomenon.

The plasma levels of DHA and androsterone in females are slightly lower than those in males. The difference, however, is not statistically significant.

A diurnal rhythm in the excretion of urinary 17-ketosteroids by men has been demonstrated by Pincus (28). A similar cycle was shown for the urinary excretion of corticosteroids (29–31), and for the plasma levels of 17-hydroxycorticosteroids (32, 33). The variation in the levels of plasma DHA is small, but significant. The variation in the concentration of steroid in blood can be considered to be the result of either: 1) a variation in production by the endocrine gland, 2) a change in the rate of destruction of steroid by the liver, or 3) a change in the rate of its excretion by the kidney. Since DHA appears to be present in the blood in a conjugated form, it is not possible at present to determine the factors accounting for the diurnal variation.

It is known that there is a tendency towards decreased urinary 17-ketosteroids in elderly individuals. Dorfman and Shipley (34) have thoroughly reviewed the data available on this question. From their compilation, it appears that the urinary 17-KS excretion of subjects between the ages of 50 and 70 years is approximately half that of subjects in the 20–30 year group, the pattern being similar in both sexes. Comparable results were obtained in our study of plasma 17-KS levels. After the age of 60 years, the plasma concentrations of DHA and androsterone decreased more rapidly than did the urinary values as reported by the foregoing authors (34). This is probably due to the fact that our micro methods for blood determinations are less accurate than the urinary techniques.

The levels of DHA and androsterone in the plasma of the umbilical cord have been shown to be comparable to those of a normal adult but lower than those of the mother of the infant concerned (9, 35). The data of Gardner (3), as well as of the present study, show that the concentration of plasma 17-KS drops rapidly to zero in the first few days of life. A similar trend has been demonstrated for urinary 17-KS excretion (36).

Values for the 2 androgens measured by the present technique appear to have a definite cycle: the levels are high at birth, decrease rapidly during the first few weeks of life, and do not rise again until the onset of puberty; they attain their maximum height between the ages of 20 and 30 years, after which they tend to decrease progressively to zero values by the age 60–70 years. Since dehydroepiandrosterone or its precursor is usually thought to be of adrenal origin, this cycle seems to be related to changes in adrenocortical function.

SUMMARY

The levels of dehydroepiandrosterone (DHA) and androsterone have been determined in the plasma of 65 normal adult males, 31 normal adult females and 22 children.

In 24 normal males, 20 to 29 years of age, the levels were respectively 48.2 ± 15.1 and 25.0 ± 10.7 μg. per 100 ml. of plasma. In 16 normal females of the same age group, the levels were slightly, but not significantly lower.

There were marked individual variations in the levels, as well as some day-to-day variation. A diurnal variation was also observed. It was comparable to, but not as marked as that observed in the plasma levels of 17-hydroxycorticosteroids.

The levels of DHA and androsterone were at their maximum between 20 and 39 years of age. After the age of 40 years, they decreased rapidly and were practically nil at 70 years. At birth the levels were similar to those found in young adults, but they rapidly fell to zero in the first few weeks of life and then rose progressively with the onset of puberty. This cycle in the levels of plasma DHA and androsterone was found in both sexes.

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A TEST FOR QUALITATIVE AND QUANTITATIVE ESTIMATION OF PREGNANE-3α,17α,20α-TRIOL-11-ONE IN URINE, AND ITS SIGNIFICANCE IN ADRENAL DISTURBANCES

MICHAEL FINKELSTEIN, PH.D. AND SARAH GOLDBERG, PH.D.

Hormone Research Laboratory, Hebrew University-Hadassah Medical School, Jerusalem, Israel

IN PREVIOUS publications we described a urinary fraction which exhibits fluorescence when heated with 85 per cent phosphoric acid (1, 2). Urine of both normal males and females contains this material. In males excretion is uniform and (calculated in terms of 17-hydroxycorticosterone) amounts to 2 mg. per twenty-four hours. On the other hand, in the urine of normally menstruating women there are usually two peaks of excretion —one at about the tenth day of the cycle and one at about the twentieth day. The mean variation during the cycle is between 0.5 mg. and 1.8 mg. per twenty-four hours. The excretion of the fluorogenic material is increased in late pregnancy to 2.5–4.0 mg. per twenty-four hours. In normal subjects excretion can be increased to four times that of the control period, by injecting 300 mg. of adrenotropic hormone in daily doses of 100 mg.

Especially pronounced was the high concentration of the urinary fluorogenic material in a case of arrhenoblastoma (4 mg. per twenty-four hours), and excretion was even higher in 2 cases of female pseudohermaphrodism due to congenital adrenal hyperplasia. In the latter 2 cases the mean excretion was from 5 to 6 mg. per twenty-four hours. From the urine of these female pseudohermaphrodites the fluorogenic material was isolated and identified as pregnane-3α,17α,20α-triol-11-one[1] (3). A rapid decline in urinary pregnanetriolone to "normal" values, as evaluated by the fluorescence reaction, was achieved by treatment of the female pseudohermaphrodites with cortisone (4). However, in a case of a virilizing adrenal tumor with high urinary fluorescence readings injection of cortisone did not influence the excretion of the fluorogenic material.

The foregoing observations provoked the following questions:

1. Is the fluorogenic material excreted by normal human subjects (male and female) identical with pregnanetriolone?
2. What is the compound which causes the fluorescence in the urine of late pregnancy?

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[1] Subsequently referred to as pregnanetriolone.

3. What is the fluorogenic compound in the urine of patients receiving corticotropin (ACTH)?
4. Does cortisone merely depress the excretion of pregnanetriolone in cases of adrenal hyperplasia or does it eliminate the excretion completely, the remaining fluorescence being due to different substances?
5. Is the excretion of pregnanetriolone specific to cases of adrenocortical hyperplasia (*e.g.*, female pseudohermaphrodism, macrogenitosomia precox), or is it also excreted in cases of virilizing adrenal tumor?
6. Is it possible to distinguish between adrenal hyperplasia and adrenal tumor on the basis of pregnanetriolone excretion?

These questions could not be answered satisfactorily so long as no analytical method was available to distinguish between pregnanetriolone and other urinary fluorogenic substances.

In this paper a method is described which allows of separation, identification and quantitative estimation of pregnanetriolone in urinary extracts. Results obtained on application of this method to several urinary extracts are reported.

EXPERIMENTAL

Detection of pregnane-3α,17α,20α-triol-11-one on paper (5)

A 5-gamma sample of crystalline pregnane-3α,17α,20α-triol-11-one in 96 per cent ethanol solution was placed on Whatman No. 1 filter paper, covering an area of no more than 1 sq. cm. The paper was dried in air and then quickly dipped in 70 per cent solution of phosphoric acid. It was blotted between two sheets of filter paper and placed on a pre-heated enamel tray in an electric oven at 87° C. for ten minutes. The tray was then cooled on ice to room temperature and viewed under an ultraviolet lamp with a maximum production of 3660 Å light (Model RX-15, Research Equipment Corporation, Oakland, California).

Under these conditions, pregnane-3α,17α,20α-triol-11-one, as well as its 3α,20α-diacetate, exhibit a blue fluorescence[2] which is clearly visible with 1 to 2 gamma of either compound.

Under the same circumstances, hydrocortisone and its 21-acetate exhibit a brilliant yellow fluorescence, produced by even less than 1 gamma of these compounds.

Paper chromatography of pregnane-3α,17α,20α-triol-11-one

Two systems of Zaffaroni (7, 8) were used for development of the chromatograms. In System I formamide:methanol 1:1 was the stationary phase and benzene the mobile phase. In System II formamide:methanol 1:1 was the stationary phase and chloroform the mobile phase. The chromatography was carried out in glass jars on Whatman filter paper No. 1 at a constant temperature of 27° C. ±1°.

In System I the solvent front reached the edge of the paper (30 cm. from the starting line) in six hours, during which time pregnanetriolone travelled about 2 cm. Usually the

[2] In the test of Neher and Wettstein (6) in which fluorescence is observed after heating paper treated with 15 per cent phosphoric acid for twenty minutes at 90° C., neither pregnanetriolone nor the diacetate exhibit fluorescence under ultraviolet light.

chromatography was carried out for twenty hours and the overflow was collected into a beaker. During that time pregnanetriolone advanced 7 cm. from the starting line.

In System II the solvent front advanced 30 cm. from the starting line in three hours. During that time pregnane-3α,17α,20α-triol-11-one moved about 10 cm. from the starting line.

Identification and quantitative estimation of pregnanetriolone in urinary extracts

Urinary extracts were prepared as described by Zondek and Finkelstein (1).

Usually, portions of 200–400 cc. from 24-hour collections of urine were taken for hydrolysis and extraction. Small portions of the final extracts were taken for fluorometry (*cf.* Zondek and Finkelstein (1)).

Aliquots of the extracts, each equivalent to 1/20 volume of the 24-hour urine specimen, were applied as evenly as possible to three paper strips of 2.5 cm. width. To the third strip, a 5-gamma sample of pregnanetriolone was added to see whether substances present in the extract interfered with the development of fluorescence by pregnanetriolone. On a fourth strip, a 5-gamma sample of crystalline pregnanetriolone was applied. The chromatograms were run in solvent System I and, after completion, one of the strips on which only the urinary extract was applied was separated from the other strips and saved. The other three strips were treated with 70 per cent phosphoric acid, and areas exhibiting fluorescence were marked. From the strip which was not dipped in phosphoric acid, a region corresponding to the position of pregnanetriolone was cut out and eluted with methanol. A small portion of the eluate (1/100 of its volume) was used for quantitative fluorometry and the remainder was divided into two parts of 90 per cent and 10 per cent of the eluate, respectively. These solutions were chromatographed in System II. On the strip with 90 per cent of the eluate, the fluorescence reaction with 70 per cent phosphoric acid was carried out and the result compared with that on a third strip treated with pure pregnanetriolone. The strip on which 10 per cent of the eluate was applied served both for elution and quantitative fluorometric estimation of pregnanetriolone. Evidence for the presence of pregnanetriolone was obtained when in both systems blue fluorescent spots were located in the position characteristic for pregnanetriolone.

Thus, well isolated regions which afforded qualitative and quantitative estimation of pregnanetriolone could be obtained from urinary extracts.

RESULTS

Urines in which the presence of pregnanetriolone was established

In 5 cases of female pseudohermaphrodism due to adrenal hyperplasia, chromatography of the urinary extracts (which became strongly fluorescent on heating with 85 per cent phosphoric acid) revealed the presence of pregnanetriolone. Treatment with cortisone reduced the excretion of the fluorogenic material to quantities seen in normal persons, but even at this lower level of excretion, the presence of pregnanetriolone was established by paper chromatography.

In 1 case of a boy with macrogenitosomia precox, conditioned by adrenal hyperplasia, the presence of pregnanetriolone could also be established. These cases are summarized in Table 1A and B.

TABLE 1. OCCURRENCE OF PREGNANETRIOLONE IN THE URINE OF PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA BEFORE AND DURING TREATMENT WITH CORTISONE

Patient	Age	Diagnosis	NF* (mg./24 hrs.)	Pregnanetriolone (qualitative) on paper	Pregnanetriolone (quantitative) after paper chromatography (mg./24 hrs.)	17-KS (mg./24 hrs.)
		A. BEFORE TREATMENT WITH CORTISONE				
M.H.	26 yrs.	Congenital adrenal hyperplasia (female pseudohermaphrodite)	5.0–6.0	Not examined	Not examined	40.0–60.0
N.K.	15 yrs.	Congenital adrenal hyperplasia (female pseudohermaphrodite)	4.0–6.0	Not examined	Not examined	40.0
S.K.	1 mo.	Congenital adrenal hyperplasia (female pseudohermaphrodite)	0.8	+	0.12	2.7– 3.6
A.R.	2 yrs.	Congenital adrenal hyperplasia (female pseudohermaphrodite	0.9	+	0.36	4.6
M.J.	6 yrs.	Congenital adrenal hyperplasia (female pseudohermaphrodite)		Not examined	Not examined	
A.M.	1½ yrs.	Congenital adrenal hyperplasia (macrogenitosomia precox)	0.87 1.2	+ +	0.11 0.24	5.4 3.0
		B. DURING TREATMENT WITH CORTISONE				
M.H.	26 yrs.	25–50 mg. daily orally, 2 yrs.	1.8 2.5	+ +	0.50 0.63	12.6 9.0
N.K.	15 yrs.	25–50 mg. daily orally, 2 yrs.	0.6 1.9	+ +	0.21 0.62	5.6 11.7
S.K.	1 mo.	15 mg. daily, 7 days	0.18	Not examined	Not examined	0.98
A.R.	2 yrs.	8 mg. daily orally, 7 days 25 mg. daily, 7 days	0.6 0.59	+ +	Not examined 0.12	3.2 4.6
M.J.	6 yrs.	7.5 mg. daily,† 64 days	1.6	+	0.4	4.0
A.M.	1½ yrs.	4 mg. daily,† 7 days 2.5 mg. daily,† 7 days	1.2 0.75	+ +	Not examined 0.07	9.0 2.5

* Neutral fluorogenic material, *i.e.*, the total fluorescence in the urinary fraction used for paper chromatography. It is calculated from the fluorescence curve of crystalline pregnanetriolone.

† Prednisone.

Urines in which the presence of pregnanetriolone was not established

Extracts of urine from normal human males (6 urines from individual subjects and pooled urine from 25 subjects) which exhibited fluorescence on being heated with 85 per cent phosphoric acid, did not show the presence of pregnanetriolone when chromatographed on paper. Also, extracts from the urine of normal women, collected on the eighth, tenth, thirteenth, twentieth, twenty-third and twenty-ninth days of the menstrual cycle did not contain detectable amounts of pregnanetriolone. Negative results were obtained with extracts from the pooled urines of late pregnancy, although

these extracts exhibited strong fluorescence when heated in a test-tube with 85 per cent phosphoric acid. In a female patient who received 300 mg. of ACTH-gel intramuscularly in doses of 100 mg. per day for three days, the fluorometric reading rose from 1.4 mg. of pregnanetriolone-equivalent in the control period, to 3.6 mg. after termination of the injections. Upon chromatography of the extract of these urines before and after treatment, no pregnanetriolone was detected.

Similarly, fluorometric analysis of the urine in a second patient who was treated with 75 mg. of cortisone acetate by mouth daily for ten days and subsequently with 200 mg. daily for three days, showed that the concentration of substances exhibiting fluorescence with 85 per cent phosphoric acid rose from 2.2 mg. on the tenth day of treatment with 75 mg. of cortisone, to 5.4 mg. on the third day of treatment with 200 mg. cortisone. No pregnanetriolone could be detected by paper chromatography in extracts of these urines. On the other hand, a spot appeared which had the same RF values as hydrocortisone and exhibited brilliant yellow fluorescence indistinguishable from that of hydrocortisone (*cf.* experimental part of this paper).

Also submitted to paper chromatography was the urine of a woman with a virilizing adrenal tumor. In several analyses the fluorescence readings were comparable to 4–10 mg. of pregnanetriolone per twenty-four hours. Spots corresponding to pregnanetriolone were not present. In a case of a woman with a non-virilizing adrenal adenoma, but with a high 24-hour urinary excretion of 17-ketosteroids (30.6 mg.) and of fluorogenic material (3.6–6.0 mg.), no pregnanetriolone could be detected by paper chromatography. These cases are presented in Table 2.

DISCUSSION

The results obtained so far clearly indicate that pregnane-3α,17α,20α-triol-11-one is excreted in cases of adrenal hyperplasia, *e.g.*, female pseudohermaphrodism and macrogenitosomia precox in males. In our series, pregnanetriolone was isolated in 2 cases (3) and identified in 6 by the test described in this paper. Fukushima *et al.* (9) recently confirmed our findings by isolating pregnanetriolone from the urine in 2 cases of adrenal hyperplasia. A further confirmation was provided by Jensen and Bjorklund (10), who found evidence of this compound in a case of female pseudohermaphrodism due to adrenal hyperplasia.

Pregnanetriolone is, however, not excreted in detectable quantities in the urine of normal men and women nor in the urine of pregnancy. Also, in a case of virilizing adrenal tumor and in a case of non-virilizing adrenal adenoma with high urinary 17-ketosteroids, there was no evidence of pregnanetriolone in the urine.

Although in some of the foregoing cases (pregnancy, and virilizing and

TABLE 2. CASES IN WHICH NO PREGNANETRIOLONE WAS FOUND IN THE URINE

Patient	Age (yrs.) & Sex	Diagnosis	NF* (mg./24 hrs.)	Pregnanetriolone (qualitative) on paper†	17-KS (mg./24 hrs.)
Ch.M.	56 F	Virilizing adrenal tumor	4.0–10.0	None	15.0–24.0
J.M.	54 F	Adrenal adenoma without virilism	3.6– 6.0	None	23.0–31.0
Pooled urine (200 women)		Pregnancy VII, ninth month	2.5– 4.0	None	
D.Z.	45 F	Normal, treated daily with cortisone:			
		75 mg. for 10 days	2.2	None	
		200 mg. for 3 days	5.4	None	
S.C.	30 F	Normal, before treatment.	1.4	None	
		ACTH, 100 mg. daily for 3 days	3.6	None	
		Normal males	1.8– 2.5	None	
		Normal females	0.5– 1.8	None	

* Neutral fluorogenic material, *i.e.*, the total fluorescence in the urinary fraction used for paper chromatography. It is calculated from the fluorescence curve of crystalline pregnanetriolone.

† No pregnanetriolone was detected in quantities above 40 gamma per 24-hour urine.

non-virilizing adrenal tumor) the fluorescence reaction with 85 per cent phosphoric acid indicated a high concentration of material similar or identical to pregnanetriolone, further analysis by means of paper chromatography showed that this fluorescence must have been due to substances different from pregnanetriolone.

Stimulation of the adrenal cortex by injections of ACTH also caused a high excretion of substances becoming fluorescent with 85 per cent phosphoric acid, due to compounds other than pregnanetriolone. Following oral administration of massive doses of cortisone acetate, the rise in material exhibiting fluorescence when heated with 85 per cent phosphoric acid could be attributed in part to the presence of 17-hydroxycorticosterone; under such circumstances pregnanetriolone was absent. However, admin-

istration of cortisone to the patients with adrenal hyperplasia lowered, but did not completely remove pregnanetriolone from the urine.

These findings suggest that in adrenal hyperplasia, steroids are metabolized in a way *qualitatively* different from that in normal individuals. Neither stimulation of the adrenals with ACTH nor administration of massive doses of cortisone gives rise to excretion of pregnanetriolone in normal subjects.

This shows that adrenal stimulation in itself is not the source of urinary pregnanetriolone, and that cortisone cannot be regarded as a precursor of pregnanetriolone.

Thus the presence of pregnanetriolone in urine seems to be specific to cases of adrenal hyperplasia, and the presence or absence of this compound may serve as a useful test to distinguish adrenal hyperplasia from adrenal tumors and from other conditions which have similar clinical manifestations.

SUMMARY

1. A test is described for *qualitative* and *quantitative* determination of pregnane-3α,17α,20α-triol-11-one in urinary extracts.

For qualitative determination, pregnanetriolone is distinguished by paper chromatography and by the blue fluorescence which it exhibits under ultraviolet light (max. 3650 Å) when the papergrams are briefly dipped in 70 per cent phosphoric acid and subsequently heated at 87° C. for ten minutes. This reaction is clearly visible with 1–2 gamma of the crystalline compound. The lowest amount of pregnanetriolone which can be detected by this test in 1/20 of a 24-hour urine specimen is 40 gamma.

For quantitative determination, areas of the paper chromatograms corresponding to crystalline pregnanetriolone are eluted and the concentration of pregnanetriolone estimated fluorometrically through the fluorescence it exhibits when heated for twenty minutes at 100° C. with 85 per cent phosphoric acid.

2. The urine of patients with adrenal hyperplasia contained pregnanetriolone.

3. Pregnanetriolone could not be detected in amounts above 40 gamma in the 24-hour urine specimens of normal persons (male and female). Neither could it be detected in the urine of pregnant women or of patients with adrenal tumor.

4. Stimulation of the adrenals with corticotropic hormone or administration of massive doses of cortisone did not provoke excretion of pregnanetriolone in normal persons. Hence pregnanetriolone cannot be regarded as a metabolic product of cortisone.

5. It is suggested that a relatively simple test, based on the *qualitative*

difference in the excretion of pregnanetriolone, could serve as an aid in differentiating between adrenal hyperplasia and adrenal tumor.

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PYRIMIDINE METABOLISM IN DIABETES MELLITUS STUDIED WITH THE URACIL TOLERANCE TEST*

RAYMOND CAREN, M.D. AND LUCILLE CORBO

The Institute for Medical Research, Cedars of Lebanon Hospital and the Department of Medicine, School of Medicine, University of California at Los Angeles, California

INTRODUCTION

THE generally accepted pathway of pyrimidine (uracil and cytosine) metabolism consists of a series of oxidations to iso-dialuric acid, rupture of the pyrimidine nucleus, and the final formation of urea (1). The chemical formulae of iso-dialuric acid and alloxan, a diabetogenic pyrimidine, are almost identical, differing only by the presence of two hydrogen atoms on the fourth carbon atom of the former. Therefore, it was postulated that during the course of pyrimidine metabolism, alloxan might form at the iso-dialuric acid stage due to an error in pyrimidine metabolism, slowly destroy the beta cells of the islets of Langerhans, and cause diabetes mellitus (2).

In previous studies uracil labeled with isotopic N^{15} nitrogen had been administered intravenously to 1 normal and to 1 diabetic subject, and the metabolic data compared (2, 3). The labeled uracil was catabolized to urea more rapidly in the diabetic. In addition an unidentified N^{15}-containing substance was excreted in the urine of the diabetic for a longer time than in the normal subject.

It then became necessary to determine whether or not a difference in uracil metabolism could be demonstrated in a large number of diabetic patients. If a characteristic difference of pyrimidine metabolism occurred, as suggested by the work with N^{15}, the abnormality should be demonstrable using a non-isotopic technique. The problem was approached by comparing the metabolism of a standard oral dose of uracil in a group of normal subjects with that in a group of diabetics. Blood uracil levels were determined at various intervals. It seemed logical to assume that if the pyrimidines were catabolized to urea more rapidly in diabetes mellitus, uracil

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would be withdrawn from the blood at a faster rate for conversion to urea by the liver. Using this "uracil tolerance test," a flat curve would be obtained if uracil were metabolized more rapidly in diabetes mellitus than in the normal state.

This report is a comparison of findings with the uracil tolerance test in normal subjects, in diabetics, and in non-diabetic patients with other illnesses. In addition, it is a presentation of a colorimetric method adapted for the determination of uracil and cytosine in blood.

METHODS

In order to carry out this work it was necessary to develop a rapid and accurate method for the quantitative determination of uracil in biologic fluids. The colorimetric method of Soodak, Pircio and Cerecedo for the determination of uracil and cytosine in pure solution (4) was modified and adapted to blood. Validity of the adaptation was assessed by a study of the sensitivity of the method, the optimum pH for carrying out the colorimetric reaction, the degree of interference by uric acid, and the recovery of known amounts of uracil added to blood.

A 2-cc. sample of Folin-Wu blood filtrate was put into an NPN tube cut down to within 1 cm. of the 25-cc. graduation mark, and acidified with 1 drop of normal sulfuric acid. After the addition of 7 drops of saturated bromine water, the solution was allowed to stand for five minutes. The excess bromine was removed by aeration. The aeration tube was washed down with 3 cc. of distilled water, followed by the addition of 5 cc. of urea-cyanide solution (25 per cent urea, 2.5 per cent sodium cyanide). Then 1.5 cc. of a 1:5 dilution of Newton's reagent (lithium arsenotungstate) was added from a microburette, and the whole was mixed with lateral shaking. The mixture was allowed to stand for one hour, diluted to the 25-cc. mark with distilled water, inverted three times using a parafilm cover, and read at 660 mμ in a Bausch and Lomb spectrophotometer (Spectronic). A blank was prepared using 2 cc. of water instead of the filtrate, and was carried through the same procedure. A 1-cc. portion of a standard uracil solution containing 0.025 mg. per cc. was diluted with 1 cc. of water and treated in the same manner.

$$\text{Calculation: } \frac{\text{O.D. (unknown)}}{\text{O.D. (standard)}} \times 0.025 \times 500 = \text{mg. uracil/100 cc. blood.}$$

The subjects were divided into three groups. There were 7 normal persons, 16 patients with diabetes mellitus, and 15 non-diabetics with other illnesses. With the exception of the normal group, all were hospitalized. All subjects fasted overnight, but were allowed to eat during the six-hour test. A blood specimen was obtained under the fasting conditions. The patient was then given 2 grams of uracil by mouth, with some water. Blood samples were collected at one, two, four and six hours, into tubes containing oxalate.

As a separate experiment, the concentration of blood uracil was determined over a six-hour period under the same conditions as those of the uracil tolerance test, but omitting the 2-gram dose of uracil. This was carried out in order to determine the effect of food on the constancy of the blood uracil level.

RESULTS

The data are presented in two parts: (a) those pertaining to the adaptation of the method for determining uracil in blood, and (b) those showing

TABLE 1. DETERMINATION OF URACIL IN PURE SOLUTIONS OF KNOWN CONCENTRATION

Uracil added (μg.)	Uracil found (μg.)
35.0	35.3
25.0	25.0
12.5	12.5
6.2	6.0
3.1	3.1
1.5	1.6

the results of uracil tolerance tests in normal, diabetic and non-diabetic patients.

The applicability of the colorimetric method of Soodak, Pircio and Cerecedo (4) was confirmed. Pure solutions of uracil produced a blue color in accordance with the Beer-Lambert Law over a range of 1.5 to 35.0 micrograms (μg.) (Table 1).

Interference by uric acid was studied next. Although the original method stated that uric acid did not yield a color reaction, we found that uric acid produced a blue color when present in large amounts—*i.e.*, exceeding 0.1 mg. (Table 2).

The degree of interference of uric acid in the presence of uracil was next determined, in order to make certain that there was no synergistic action when they were present in the same solution. As expected on the basis of the foregoing data, uric acid did not interfere until it was present in quantities exceeding 0.1 mg. (Table 3). Thus, in the case of blood, uric acid will

TABLE 2. DEGREE OF COLOR PRODUCED BY URIC ACID

Uric acid (mg.)	Optical density
0.2	0.02
0.2	0.02
0.1	0.00
0.1	0.00

TABLE 3. DEGREE OF COLOR PRODUCED BY URIC ACID IN THE PRESENCE OF URACIL

Uracil added (μg.)	Uric acid added (mg.)	Optical density
22.4	0.00	0.19
22.4	0.10	0.18
22.4	0.15	0.18

TABLE 4. INFLUENCE OF pH ON OPTICAL DENSITY, WITH CONSTANT QUANTITY OF URACIL

pH	Optical density
8.0	0.21
7.5	0.33
7.1	0.47
2.9	0.49
2.3	0.51

not interfere with the determination of uracil until it exceeds a concentration of 100 mg. per 100 cc. Since such concentrations, or even half this amount, are not found in blood, uric acid can be disregarded in this determination.

A full-strength solution of lithium arsenotungstate (Newton's reagent) was used in the original method of Soodak, Pircio and Cerecedo. This was found to produce a color that was too dark. A 1:5 dilution of the reagent yielded the most satisfactory results.

The most important modification involved the pH at which the bromination was performed. In the original method, this step was carried out in a neutral medium, and no mention was made of pH. Maximum color was obtained when the bromination occurred in an acid medium (Table 4); consequently, the pH was adjusted to the acid side with 1 drop of normal sulfuric acid just before bromination.

The Folin-Wu method of preparing a protein-free filtrate was found to be most satisfactory for the determination of blood pyrimidines. Experiments were conducted on the recovery of known amounts of uracil added to blood. The initial blood uracil level was subtracted from the level found after addition of uracil. In each case the difference agreed well with the amount added (Table 5).

Table 6 lists the results of uracil tolerance tests in 7 normal persons. Fol-

TABLE 5. RECOVERY OF KNOWN AMOUNTS OF URACIL FROM BLOOD

Initial blood uracil level (mg./100 cc.)	Uracil added (mg./100 cc.)	Total uracil (mg./100 cc.)	Uracil recovered (total minus initial) (mg./100 cc.)	Per cent recovery
0.82	5.00	6.30	5.48	110
0.62	2.50	3.10	2.48	99
0.62	1.25	1.90	1.28	102

TABLE 6. RESULTS OF URACIL TOLERANCE TESTS IN NORMAL SUBJECTS FOLLOWING AN ORAL DOSE OF 2 GRAMS OF URACIL

	Level of blood uracil (mg./100 cc.)				
	0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.
L.C.	0.55	3.10	4.95	1.51	0.60
C.H.	0.50	2.93	2.93	1.51	2.88
C.M.	0.80	3.80	3.04	1.08	0.56
D.K.	0.56	2.90	2.10	1.17	0.70
D.H.	0.95	1.88	2.85	2.23	0.62
M.N.	0.85	2.32	2.08	1.43	0.95
S.	1.31	2.10	1.99	1.84	1.05

lowing the 2-gram oral dose of uracil, the blood uracil level rose to a peak value by either the first or second hour and averaged 2.22 and 2.84 mg. per 100 cc., respectively. The values then fell slowly to the control level by the end of the sixth hour. In the case of C.H. the six-hour value remained elevated and was higher than the four-hour value. This persistent elevation did not occur in any other normal subject, or in the diabetic and non-diabetic patients. The control uracil levels varied from 0.50 to 1.31 and averaged 0.79 mg. per 100 cc.

The concentration of blood uracil remained fairly constant in 2 normal subjects during the six hours of observation, despite the ingestion of food, nor was any significant change found in the concentration two hours after eating in 3 patients studied (Table 7).

Table 8 lists the results of uracil tolerance tests in 16 diabetic patients. The control values averaged 0.84 mg. of uracil per 100 cc. of blood and were about the same as in normal subjects. The height and shape of the individual curves closely resembled those of the curves for the normal group.

TABLE 7. THE EFFECT OF FOOD ON THE BLOOD URACIL LEVEL

	Level of blood uracil (mg./100 cc.)				
	0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.
L.C.*	0.94	0.63	0.63	0.63	0.63
R.C.*	0.63	0.94	0.94	0.63	—
R.G.	1.00	—	1.00		
M.L.	1.00	—	1.50		
M.B.	1.00	—	1.00		

* Normal subjects.

The peak value occurred in one or two hours and averaged 2.56 and 2.35 mg. per 100 cc., respectively. As in the normal subjects, a plateau was reached at this point and the concentration of uracil fell to the control level by the sixth hour.

Three diabetics (S.K., J.C. and M.R.) had flat uracil tolerance curves. In 1 of these (S.K.), the clinical diagnosis was intercapillary glomerulosclerosis. In 2 other patients with the same diagnosis, the curves were of the normal type. Insulin did not appear to have any influence on uracil tolerance.

TABLE 8. RESULTS OF URACIL TOLERANCE TESTS FOLLOWING AN ORAL DOSE OF 2 GRAMS OF URACIL IN 16 PATIENTS WITH DIABETES MELLITUS

Patient	Level of blood uracil (mg./100 cc.)					Dose of insulin	Additional diagnosis
	0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.		
R.G.	1.00	4.00	3.10	1.60	0.95	0	Arteriosclerotic heart disease
S.K.	0.57	0.52	0.52	1.40	0.82	10 NPH	Intercapillary glomerulosclerosis and retinopathy
D.J.	0.94	1.78	2.26	0.78	0.50	0	Hypertension
J.C.	1.00	1.15	1.27	1.15	1.55	60 NPH	Asthma
J.B.	1.00	2.20	1.37	1.14	1.32	0	Hodgkin's disease
L.S.	0.59	3.97	2.98	0.50	0.50	10 NPH	Uterine fibroid
M.R.	1.05	1.67	1.34	1.09	0.46	30 reg.	Asthma
G.M.	0.31	3.10	2.70	0.50	0.31	0	Arterial insufficiency
A.M.	0.50	0.56	2.30	1.16	0.50	38 NPH	Arteriosclerotic heart disease
L.W.	0.64	1.03	2.46	2.02	—	30 NPH	Thyroid nodule
E.A.	0.65	3.80	4.40	3.37	1.03	10 NPH	Arteriosclerotic heart disease
R.A.	0.89	2.01	2.21	1.79	0.99	30 NPH	Intercapillary glomerulosclerosis and retinopathy
R.B.	1.12	3.47	2.18	1.11	0.89	30 NPH	Cholecystitis
F.S.	0.88	3.34	3.48	1.72	0.88	0	Arteriosclerotic heart disease
M.B.	1.47	5.32	3.68	1.47	1.03	25 NPH	Arteriosclerotic heart disease
E.L.	0.80	3.06	1.34	0.67	0.67	20 NPH	Intercapillary glomerulosclerosis—Received 10 mg. of Meticorten

Table 9 lists the results of uracil tolerance tests in a group of 15 patients with diseases other than diabetes mellitus. The average control value was 0.88 mg. of uracil per 100 cc. of blood—about the same concentration as in the other two groups of subjects. In general, the curves were similar in height and shape to those of the other two groups. Five patients (S.G., J.E., L.P., R.Z. and H.C.) had flat uracil tolerance curves; in fact, the curves were almost straight lines. Three of these 5 patients had received ACTH or cortisone at the time the test was made. However, flat curves were also observed in the case of 2 patients who had not received these substances; therefore this type of curve could not be attributed to ACTH or cortisone. Further corroboration was demonstrated by the results in Patient A.B., who received ACTH and responded with a normal curve. Because of the 5 flat curves, the composite curve of this group was lower than the composite curves of the normal and diabetic groups (Fig. 1).

TABLE 9. RESULTS OF URACIL TOLERANCE TESTS FOLLOWING AN ORAL DOSE OF 2 GRAMS OF URACIL IN 15 PATIENTS WITH DISEASES OTHER THAN DIABETES MELLITUS

Patient	Level of blood uracil (mg./100 cc.)					Diagnosis
	0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.	
F.S.	0.97	4.40	3.70	1.00	1.00	Congestive heart failure
G.F.	0.92	4.30	2.80	2.60	2.10	Herniated disc
M.G.	0.80	2.90	2.50	1.70	1.30	Arteriosclerotic heart; congestive failure
E.G.	1.05	1.94	2.28	1.72	1.10	Virus pneumonia
A.S.	0.64	0.98	0.88	2.64	1.96	Fusion of hip
D.W.	1.76	2.48	2.62	1.91	1.00	Endometriosis
D.W.	0.82	1.27	1.27	1.59	0.87	Congestive heart failure
S.G.	1.32	1.32	1.65	1.26	1.04	Arteriosclerotic heart disease
J.F.*	0.63	0.90	0.68	0.90	0.68	Monocytic leukemia
E.S.	0.83	2.50	3.00	2.20	1.38	Arteriosclerotic heart disease
L.P.*	0.69	1.27	0.74	0.74	1.13	Carcinoma of thyroid
R.Z.	0.20	0.50	0.99	0.99	0.99	Arteriosclerotic heart disease
H.C.*	0.93	0.70	1.29	1.02	1.29	Myasthenia gravis
S.M.	0.60	0.60	—	1.53	0.83	Serum hepatitis
A.B.*	0.94	3.75	3.06	1.25	1.12	Papilledema; cause unknown

* Received cortisone during the test.

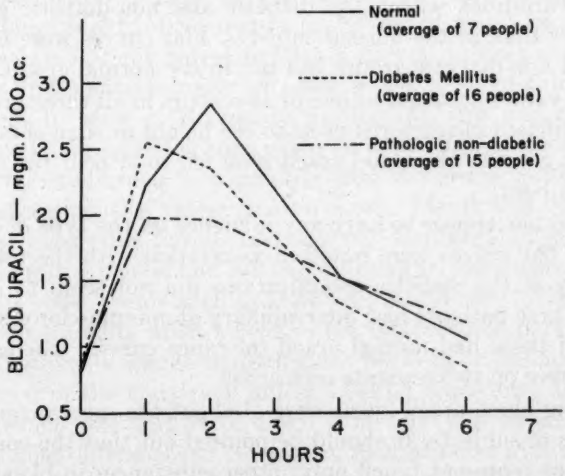

FIG. 1. Uracil tolerance curves following ingestion of 2 grams of uracil, in normal subjects, diabetics, and non-diabetic patients with other diseases. (See text for explanation of flat curve in third group.)

DISCUSSION

No alteration of pyrimidine metabolism was found in diabetes mellitus, as measured by the uracil tolerance test. Comparison of the uracil tolerance curves of normal subjects, diabetic, and patients with non-diabetic illnesses did not reveal any characteristic differences.

The criteria adopted for a normal tolerance curve consisted of a rise in the blood uracil level to at least 2.0 mg. per 100 cc. in one or two hours with a return to the normal level ± 0.3 mg. per 100 cc. by the sixth hour. According to these criteria there were 3 abnormal curves in the diabetic group and 7 in the non-diabetic pathologic group. However, there were only 7 subjects in the normal group as compared with 16 in the diabetic and 15 in the non-diabetic pathologic groups. The percentage of abnormal curves would have to be much greater in the diabetic group than in the non-diabetic group to be of any significance. The 3 abnormal curves in the diabetic group did not constitute sufficient evidence for concluding that a characteristic abnormality of pyrimidine metabolism occurs in diabetes mellitus. The abnormal curves represented individual variations within each group and were not characteristic for that group.

Upon examination of the individual curves of each group, considerable variation was noted in the height of the blood uracil level at any given time. There were flat curves which never attained a height of 1.0 mg. per 100 cc. and elevated curves which reached a height of 5.0 mg. per 100 cc. Individual variations within the diabetic and non-diabetic groups were much greater than in the normal subjects. Flat curves were found in the diabetic and non-diabetic groups but not in the normal group.

The peak values occurred at one or two hours in all three groups. There were no significant characteristics as to the height or time of occurrence of the peak. In general, the blood uracil level fell to or near the control level by the sixth hour.

Insulin did not appear to have any influence on the type of curve. Both normal and flat curves were noted in association with the use of insulin. The severity of the diabetic complications did not seem to affect uracil tolerance. Three patients had intercapillary glomerulosclerosis with retinopathy; 2 of these had normal uracil tolerance curves, whereas the third had a flat curve on two separate occasions.

The fasting blood uracil levels were of about the same magnitude in all three groups of subjects. It should be pointed out that the control fasting level does not represent uracil only; other substances in blood contribute to the color reaction, such as cytosine (pyrimidine) and guanine (purine). Cytosine produces about the same color intensity as an equal amount of uracil, whereas guanine produces about one tenth the color per unit of weight. The nucleotides of these bases also give rise to a faint color reac-

tion. It should be emphasized that although there are other substances in blood which produce the color reaction, the fasting or control level of blood uracil was shown to remain constant (varying only ±0.3 mg. per 100 cc., and uninfluenced by food) during the six-hour test period (Table 7); hence the intensity of color was considered to represent uracil only. Fluctuations of the blood uracil level during the test were due only to the 2-gram dose of uracil and were accurately determined by the method. Uric acid was not present in sufficient quantities to alter the color reaction. Thus, although the method for determining uracil as described in this paper is not completely specific, it proved satisfactory as a measure of pyrimidine metabolism by permitting comparison of blood uracil levels.

It is interesting to speculate why there were such great individual variations in the uracil tolerance curves in the diabetic and non-diabetic pathologic groups. Possibilities that must be considered are differences in the rate of urinary excretion and in the rate of absorption from the gastro-intestinal tract. Horrigan reported a 24-hour urinary excretion of 126 mg. (6.3 per cent of the dose) in normal people following the oral dose of 2 grams of uracil (5); the uracil was isolated on a chromatographic column and determined by ultraviolet absorption. However, there are no adequate data regarding these factors at the present time.

The ultimate fate of approximately 50 per cent of ingested uracil remains obscure. Previous work has shown that not all of a given dose of uracil is metabolized to urea (2, 3). Only 50 per cent of isotopic heavy nitrogen administered intravenously as N^{15}-uracil could be recovered in the urine over a 72-hour period in both a normal subject and a diabetic. During the first eighteen hours an unidentified N^{15}-containing substance was excreted. In view of Horrigan's finding (5), this was probably unchanged uracil. The question then arises as to the fate of the remaining 50 per cent of ingested uracil. It is unlikely that this uracil becomes a part of the pyrimidines of the tissue nucleic acids. Plentl and Schoenheimer showed that exogenous pyrimidines are not incorporated into the body nucleic acids of the pigeon and rat (6), differing in this respect from other normal tissue constituents such as amino acids and fatty acids. Most normal tissue constituents when added to the diet become part of the body tissues, so that one is unable to distinguish between exogenous and endogenous material in the body pool unless labeling procedures are used. The manner in which this portion of uracil is metabolized remains unknown. Thus, individual differences in the rate of utilization of pyrimidines is the most likely explanation for the various types of uracil tolerance curves.

SUMMARY AND CONCLUSIONS

1. There is no alteration in pyrimidine metabolism characteristic of diabetes mellitus, as measured by the uracil tolerance test.

2. Abnormal uracil tolerance curves are frequent, both in patients with diabetes and those with other diseases.

3. A successful adaptation of a colorimetric method for the determination of uracil in blood is presented.

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INACTIVATION OF ANTIDIURETIC SUBSTANCE BY HUMAN LIVER

HAJIME IMANAGA, M.D., TATSUHEI KONDO, M.D.* AND ISAMU MORI, M.D.

Department of Surgery, Nagoya University School of Medicine, Nagoya, Japan

VARIOUS elements have been considered as causing edema or ascites in patients with hepatic cirrhosis, and especially has antidiuretic substance been considered to be a factor in the production of ascites in liver disease. Since Malaguzzi-Valeri *et al.* pointed this out in 1941 (1), studies have been made by many others, among them Ralli *et al.* (2), Lloyd and Lobotsky (3), Perry and Fyles (4) and Goldman and Luchsinger (5). The ayes and nays are about equally divided, and there is still no true identification. In this paper is presented a study which the authors recently undertook in an effort to clarify the decreased inactivation of antidiuretic substance in the liver of patients suffering from portal hypertension. Liver fragments were removed for *in vitro* study, and then antidiuretic substance was injected into the portal vein.

METHODS

Measurement of antidiuretic activity (ADA)

Various biologic methods have been used for this purpose; we selected that of Jeffers and associates (6) which is said to be most sensitive. Male adult rats were used each weighing 150–200 Gm., all bred in the same circumstances with a diet of solid food and unlimited water. A volume of 12 per cent ethanol-water solution equivalent to 5 per cent of the body weight and previously warmed to body temperature is forced into the stomach. After thirty minutes (with the rat in a narcotized condition) a volume of warm water equivalent to 3 per cent of the body weight is introduced in the following way: The rat is fixed on a board on its back. By vesicotomy, a fine vinyl tube is inserted into an incision made in the urinary bladder, and the urethra is bound. For the first twenty minutes, the volume of urine is measured every five minutes as it drops through the vinyl tube. When the excretion is the same for each five-minute period, 0.2 ml. of the material to be tested is injected into the femoral vein. For the second twenty-minute period, the volume of urine is measured every five minutes and the total volume compared with the total volume during the first twenty-minute period before injection. Thus the ratio between the two periods is found.

If a represents the urinary volume in milliliters before injection and b represents the volume after injection, then $(a-b)/a$ represents the antidiuretic activity (ADA) of the material tested. In biologic experiments using various kinds of samples, b is sometimes

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* Present address: Roswell Park Memorial Institute, 666 Elm Street, Buffalo 3, N. Y.

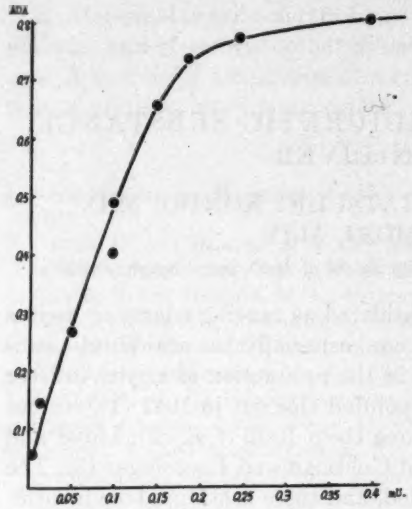

FIG. 1. Antidiuretic activity of Pitressin.

greater than *a*; in such an instance, there is a minus value for ADA. Pitressin is used for the standard. Pitressin units per 0.2 ml. parallel the ADA up to 0.15 milliunits (mU.) (Fig. 1).

Administration of Pitressin through the portal vein

When a patient with portal hypertension or other hepatic disease underwent laparotomy, 1,000 mU. of Pitressin was injected into the superior mesenteric vein and thus carried directly to the liver through the portal vein. A sample of blood was taken from the cubital vein at regular intervals to measure serum ADA. As the ADA of blood is easily influenced by various body conditions, this procedure was carried out only when we could measure portal pressure at laparotomy. Blood samples were taken before injection of Pitressin and fifteen, thirty, forty-five and sixty minutes thereafter. The serum was separated immediately, and the ADA was measured on a 0.2-ml. aliquot.

In vitro *inactivation of Pitressin by liver tissue*

At laparotomy, about 1.0 Gm. of liver tissue was resected. It was immediately homogenized in cold water and, after centrifugation, 1.0 ml. of the supernatant liquid was mixed with 3.0 ml. of phosphate buffer (pH 6.8) and 1.0 ml. of Pitressin solution containing 2.5 mU. The mixture was incubated for thirty minutes at 37° C., and 0.2 ml. was used as material for determination of ADA.

RESULTS

Inactivation of Pitressin by the liver when injected through the portal vein

The study was conducted on 17 patients—6 with no portal hypertension and no ascites, 5 with portal hypertension and no ascites, and 6 with both portal hypertension and ascites (Table 1).

In the 6 patients with no portal hypertension, before the hepatic injection of Pitressin, the average serum ADA value was −0.07. Fifteen minutes after injection of 1,000 mU. of Pitressin through the portal vein, the average ADA value for the 6 cases was +0.03. According to our method, the average value for serum ADA in the normal human subject not loaded with Pitressin is below +0.15. Thus, it may be seen that in these 6 cases the Pitressin was immediately inactivated and the serum ADA level was within normal limits.

In the 11 cases of portal hypertension with and without ascites, thirty minutes after the injection of Pitressin the serum ADA value was normal

TABLE 1. THE ANTIDIURETIC ACTIVITY (ADA) OF BLOOD FROM CUBITAL VEIN AFTER LOADING THE LIVER WITH 1,000 MU. OF PITRESSIN THROUGH THE PORTAL VEIN

Patients	Antidiuretic activity of serum*					Ascites
	Before injection	After injection of Pitressin				
		15 min.	30 min.	45 min.	60 min.	
With portal hypertension						
S.K.	+0.11	+0.51	+0.38	+0.11	—	Yes
K.H.	−0.10	−0.06	+0.14	−0.05	+0.08	Yes
O.B.	−0.12	+0.06	+0.09	+0.25	0	Yes
K.M.	−0.10	0	+0.23	+0.25	−0.10	Yes
I.M.†	−0.01	−0.03	+0.14	+0.23	+0.18	Yes
K.M.	+0.16	+0.08	+0.28	+0.25	—	Yes
Average	*−0.01*	*+0.09*	*+0.21*	*+0.18*	*+0.04*	
M.K.†	−0.06	0	+0.17	+0.27	+0.25	No
A.S.†	−0.07	+0.21	+0.24	+0.04	−0.05	No
O.H.†	−0.02	+0.17	+0.27	+0.15	−0.12	No
D.B.†	0	+0.07	0	−0.05	+0.06	No
T.T.	−0.05	−0.01	+0.02	−0.01	−0.05	No
Average	*−0.04*	*+0.09*	*+0.14*	*+0.08*	*+0.02*	
Without portal hypertension						
No. 1	−0.06	—	+0.08	0	0	No
2	−0.06	+0.14	−0.10	+0.02	+0.06	No
3	+0.05	—	+0.04	−0.08	—	No
4	−0.09	0	−0.04	+0.11	+0.08	No
5	−0.12	−0.05	−0.12	−0.19	−0.16	No
6	−0.14	—	+0.08	−0.10	−0.11	No
Average	*−0.07*	*+0.03*	*−0.01*	*−0.04*	*−0.03*	

* Plus and minus signs indicate values respectively above and below the zero line.
† Cases of obstruction in portal vein or with strong collateral circulation.

in 5, but over +0.15 in the other 6. The overall average for the 11 cases was +0.18—a rise which indicated the decreased inactivation of the Pitressin load in this group compared with patients having no portal hypertension.

Among the 11 patients suffering from portal hypertension, a comparison was made between those with ascites and those without ascites. In 3 of the 6 patients with ascites, the serum ADA was less than +0.14 thirty minutes after injection of Pitressin, *i.e.*, within the high-normal range; in the other 3 it was considerably higher. In 2 of the 5 patients with no ascites, the serum ADA was low (near zero), and in the other 3 was somewhat high. In the latter 3 cases there was either an obstruction in the portal vein or a

strong collateral circulation, both of which are thought to play the same role as Eck's fistula.

On the average, in cases with portal hypertension and ascites, the serum ADA value thirty minutes after injection of Pitressin was +0.21, and in cases without ascites it was +0.14—almost within normal limits. It can therefore be said that in the cases of portal hypertension with ascites, the inactivation of an hepatic load of Pitressin was definitely decreased and in cases with no ascites, inactivation was carried out normally so that serum ADA values fell to a normal level.

In vitro *inactivation of Pitressin by liver tissue*

In cases with no portal hypertension, and with neither liver degeneration nor ascites, inactivation was strong and the average ADA value of 0.1 mu. of Pitressin was decreased to about +0.1, from +0.5.

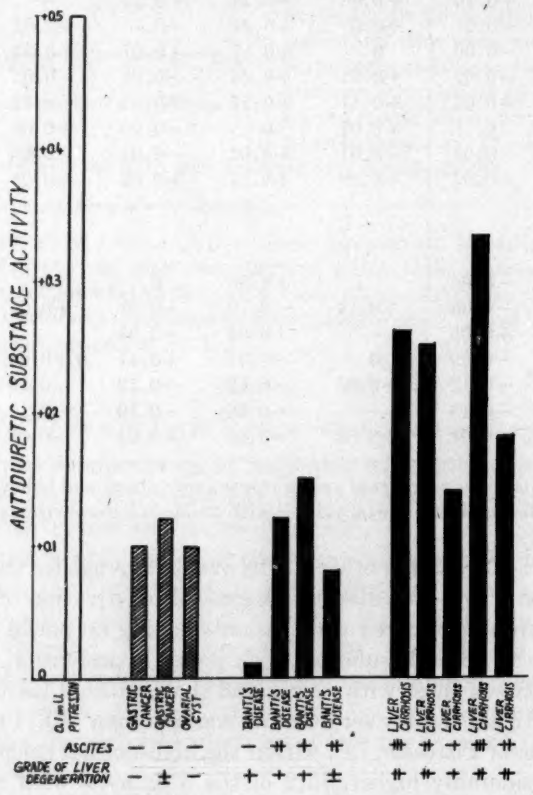

FIG. 2. *In vitro* inactivation of Pitressin (0.1 mu.) by liver tissue from patients with various diseases.

Among the patients with portal hypertension were some with Banti's disease; in these, the liver was only slightly or not at all degenerated and Pitressin inactivation was almost normal even when ascites was present. However, among patients with portal hypertension having liver cirrhosis, the liver was markedly degenerated, inactivation of Pitressin by liver tissue was decreased, and the ADA of experimental fluid was high after incubation. The more degenerated the liver, the greater was the tendency toward reduced inactivation of Pitressin by liver tissue (Fig. 2).

DISCUSSION

Eversole, Birnie and Gaunt (7) injected Pitressin directly into rats intrasplenically, intrahepatically, and subcutaneously and observed the state of water excretion. They stated that the liver is one site of inactivation of the antidiuretic substance, and that in cases of liver insufficiency, the effect is less strong and parallels the ascites. Baez, Mazur and Shorr (8) suggested another element, ferritin, as the cause of ascites. Then Birnie (9) ascertained that Pitressin is inactivated by rat liver, spleen, kidney, muscle and blood *in vitro*, and that especially in rat liver, there is some enzymatic substance which causes marked inactivation. Pitressin is also inactivated by human liver. According to Birnie, this capacity of the liver is decreased in chloroform poisoning or in adrenalectomized animals. Osono (10) experimentally bound the artery to dog liver and verified *in vitro* that inactivation of antidiuretic substance was proportionate to the degree of liver degeneration.

On the contrary, there have been many reports that liver insufficiency does not decrease inactivation of antidiuretic substance. For example, White *et al.* (11) injected physiologic amounts of Pitressin into the veins of patients with liver insufficiency, and could find no loss of hepatic inactivation. Nelson and Welt (12) injected various amounts of Pitressin and also could not find any distinctive difference. Bernstein *et al.* (13) injected Pitressin intravenously in cases of cirrhosis of the liver, and disproved any unusual inactivation of Pitressin in this disease. Miller and Townsend (14) conducted experiments on the *in vitro* inactivation of Pitressin by the livers of rats, of normal human beings, and of patients with liver diseases, and could find no significant difference among them. They therefore concluded, relative to ascites in patients with hepatic cirrhosis, that the reduction in inactivation of antidiuretic substance was not the factor, but rather overproduction of antidiuretic substance or the effects of ferritin.

Our experimental methods differed from those of previous reports. We injected 1,000 MU. of Pitressin into the portal vein of normal human subjects and of patients with portal hypertension, especially those with ascites, and found the ADA of blood from the cubital vein to be high, at thirty

or forty-five minutes after injection. This difference was due to loss by the liver of its power to inactivate a Pitressin load supplied through the portal vein. That is to say, inactivation of Pitressin by the liver was decreased in the patients suffering from portal hypertension, especially in those with ascites.

This fact was also proved by *in vitro* experiments. Moreover in the highly degenerated state of the liver tissue in cases of hepatic cirrhosis, the reduction in the ability of the liver to inactivate Pitressin is especially remarkable.

SUMMARY

1. After injection of 1,000 mU. of Pitressin into the portal vein at laparotomy, the antidiuretic activity of cubital vein blood is remarkably strong in patients with portal hypertension, especially in those with ascites.

2. In *in vitro* experiments, there is weak inactivation of Pitressin by liver tissue from patients with ascites. The effect is especially weak in liver tissue from patients with hepatic cirrhosis, a condition in which hepatic degeneration is severe.

3. Thus, in patients with portal hypertension, it would seem that a decrease in inactivation of antidiuretic substance by the liver is an important factor in the formation of ascites.

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EFFECT OF RE-USE OF *RANA PIPIENS* UPON THE ACCURACY OF THE PREGNANCY TEST

RUSSELL HILF, PH.D. AND IRVING ROSEN, M.S.*
Sixth U. S. Army Medical Laboratory, Fort Baker, California

INTRODUCTION

VARYING degrees of accuracy have been reported in using *Rana pipiens* as a pregnancy test animal (1–5). In all of these reports, the frogs were employed for only one determination. Indeed, Cutler (6) has maintained that *R. pipiens* cannot be used with reliability after the first test, if the result has been positive. Thorberg (7), on the other hand, has shown that *Bufo bufo* and *R. esculenta* can be used over again without loss of accuracy. The data presented here demonstrate the accuracy which was obtained using *R. pipiens* as many as five times for pregnancy testing.

METHODS

I. *Maintenance of frogs*

From 8 to 10 new frogs as received from suppliers[1] were placed in 8″×8″ battery jars containing 1 inch of tap water to which powdered sulfadiazine was added. The jars were immediately placed in a refrigerator set at 6° C. and kept under constant illumination by a 100-watt unfiltered Mazda bulb (8). Frogs were removed from the refrigerator as needed and placed in dry battery jars during the test period.

Upon completion of the test, 1 inch of tap water was added to the jar and the frogs were left at room temperature for ten days. At the end of this period, fresh sulfadiazine was added and the frogs were returned to the illuminated refrigerator until used again. This re-use was not sooner than four days. Water was changed daily in the jars kept at room temperature but only added when necessary to the jars in the refrigerator. No food was offered to the animals at any time.

No check was made on the refractoriness of new frogs to chorionic gonadotropic hormone (CGH), as this was accomplished during the course of the pregnancy test itself. Occasionally, however, such a procedure was followed when the source of supply changed or there was some reason to doubt the sex of the animal. In these cases the frogs each received an injection of 50 I.U. of CGH[2] and were observed as in a regular pregnancy test. New frogs were checked as to their sex by external differences—the enlarged thumb pad and vocal sacs.

II. *Urine*

To eliminate non-first morning specimens, urines with a specific gravity of less than 1.012 or a pH of over 9.0 were rejected. A 50-ml. aliquot of urine was placed in a glass-

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* Present Address: Department of Zoology, Columbia University, New York, N. Y.

[1] E. C. Steinhilber, Oshkosh, Wisconsin; Marcus & Co., San Francisco, California.

[2] Antuitrin "S," Parke, Davis & Co.

stoppered cylinder and brought to pH 4.0–4.5 with 25 per cent HCl. After the addition of 10 ml. of 5 per cent kaolin suspension, the mixture was shaken and placed in the refrigerator overnight. From 30 to 35 ml. of the supernatant material was discarded. The remaining sediment was shaken to effect suspension, placed in a 50-ml. centrifuge tube, and centrifuged at 2,000 rpm for ten minutes. The supernatant fluid was discarded, and 5 ml. of 0.1*N* NaOH added to the sediment. The precipitate was stirred and recentrifuged. The resulting supernatant material was poured into a 50-ml. beaker, a drop of 1 per cent phenolphthalein added, and the solution made neutral with 25 per cent HCl.

III. *Serum*

Serum sent for testing was injected without prior processing. It was found that a minimum of 1 ml. was required to insure accuracy.

IV. *Pregnancy test*

Two frogs were used for each test, and whenever possible from the same refrigerated jar. When this was not possible, frogs were chosen so that each of the partners had been used the same number of times and had been placed in the refrigerator at approximately the same time. A 2-ml. sample of the neutralized urinary concentrate was injected into the dorsal lymph sac of each frog, using a 2-cc. syringe and a #23-gauge needle.

Frogs were checked at one hour and at three hours from the time of injection for the presence of sperm in the urine. Slides were examined under the low-power objective, without a cover slip, in order to insure motility of the sperm. This was found to be essential when only a few sperm were present. Often it was difficult to distinguish between a non-motile sperm cell and a piece of debris or a crystal washed from the cloaca. Results of the pregnancy test were considered positive if at any time during the two examinations one or both frogs showed any motile sperm.

To minimize the possibility of errors due to refractory animals, frogs demonstrating a negative response after both checks received an injection of 50 I.U. of CGH and were checked again in one hour. The presence of sperm in either or both of the frogs indicated a negative result. Frogs showing no response to the CGH injection were considered refractory and discarded.

The frogs were not checked for the presence of sperm before their use. Under the conditions of the test it was felt that sperm present from a previous test would have disappeared by the time the animals were used again (7, 9). This assumption has been borne out by the results wherein no false positive findings were obtained.

At the completion of the experiment, information about the patient was obtained regarding the final diagnosis, last known menstrual period, expected dates of confinement and related data.

RESULTS AND DISCUSSION

During these experiments, 962 frogs were tested. Among the 485 frogs used for the first time, 251 frogs were used a second time; 125 frogs of this latter group were used for a third test; 79 frogs of this group, in turn, were used a fourth time; and finally, 22 frogs were used for a fifth test. Thus, the total number of pregnancy tests was 481. However, only 424 reports were received, from which information could be extracted. Of these 424 tests, 171 results were reported as correct positives and 174 as correct negatives —a total of 345 correct results (Table 1). On the basis of adjustments, as

outlined in the following paragraphs, the final total number of cases was 359, and the accuracy of the test was 96 per cent (Table 1).

Because of the uselessness of giving credence to information that is not entirely complete, reports which indicated that the patient had aborted were excluded from the final tabulation. There were 50 abortions and ectopic pregnancies (Table 1); in 21 abortions and 1 ectopic pregnancy the results were positive, and in 24 abortions and 4 ectopic pregnancies the results were negative. Whenever dates of abortion were reported, results of tests conducted prior to that date were always positive. Specimens collected after the date of abortion yielded negative findings. However, all of

TABLE 1. RESULTS OF PREGNANCY TESTS, USING *Rana Pipiens* AS THE TEST ANIMAL

Total reported tests			424
Ectopic pregnancy	5*		
Abortion	45*		
Correct positive results		171	345
Correct negative results		174	
Possible errors	29†		
Adjusted total errors			14
Adjusted total reported tests			359
Accuracy‡			*96%*

* Not included in determination of accuracy.

† When tests on specimens collected earlier or later than 30–90 days after conception were subtracted, the result was the adjusted total errors.

$$\ddagger \frac{\text{Adjusted total reports—adjusted total errors}}{\text{Adjusted total reports}} \times 100$$

the foregoing 50 cases were eliminated from the total reportable results, leaving 374 cases among which 29 possible errors were noted.

Smith, Albert and Randall (10) reported that CGH appears in the urine and serum of normally pregnant women on or about the fifth day before the onset of the first missed menstrual period, or from ten to twelve days after conception. The level at this time is extremely low and remains this way for from twenty-four to forty days—titers of CGH during this period being between 1,000 and 5,000 I.U. per 24-hour urine volume. During the period from forty to seventy days, there is a constant increase in hormone concentration, with a peak at approximately seventy days and a titer between 100,000 and 450,000 I.U. From seventy to ninety days there is a steady decline, until a level of 2,000 to 15,000 I.U. is attained; this continues until the one hundred and twentieth day.

Kontaxis (8) determined that the 100%-response level for *R. pipiens* under similar conditions at this laboratory was 40 I.U. of chorionic gonadotropin. From twenty-four to forty days after conception, the level of hormone in the urine is 1,000–5,000 I.U. per 24-hour specimen. Estimating that an average 24-hour volume is 1,000 ml., the concentration of hormone would be from 1 to 5 I.U. per ml. The procedure used in these experiments requires a minimum 50-ml. aliquot of urine which is concentrated to 5 ml. A 2-ml. aliquot of this concentrate is injected, so that each frog theoretically receives 20–100 I.U. of CGH. The same would be true for specimens collected ninety days after conception. Specimens obtained between these times contain a greater amount of CGH per milliliter, and a possible maximum of 9,000 I.U. might be injected into a test animal. Although a 50 per cent loss might occur during the concentration procedure, the accuracy of the results obtained shows that enough CGH remains to produce an adequate response in the test animals. In our series, urine specimens were acceptable if collected from thirty to ninety days after suspected conception. In this way, errors due merely to early or late collections were eliminated. Employing the foregoing criteria, 15 cases were eliminated from the 29 possible errors because the urine specimens were collected either too early or too late[3] (adjusted total errors—Table 1).

Three of the eliminated cases proved to be of interest. In 1 case, a negative result was obtained from a urine specimen submitted five months after conception. Since this exceeded the time limits, it was not considered a true error. However, it was subsequently noted that another test had been run on the same patient one month after the aforementioned test, or six months after conception. The results were positive.

The second case involved 2 urine samples that were submitted on 1 patient approximately thirty days apart. With the first sample, there was a negative response; it had been collected fifteen days after conception, and was ruled out as having been obtained at too early a date. The second specimen was submitted forty-five days after conception, but again there was a negative result, which was considered to be a true error.

In the third case there was an erroneous result involving a serum specimen submitted two months after conception. In this instance only 0.85 ml. of serum could be injected into each frog. Because of the small quantity of material, the result in this case was not considered a part of the final total.

Fourteen tests had false negative results (adjusted total errors—Table

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[3] Dates of conception were calculated from the first day of the last normal menstrual period.

1). In all of these cases, no false positive results were obtained and all the animals used responded to CGH at the conclusion of the test. Since all non-responding animals were eliminated by this procedure, refractoriness alone could not account for these errors—some of which merit special attention.

In one case described previously, two tests performed on the same patient at intervals of fifteen and forty-five days after conception yielded negative results. In a second case, urine and serum samples were submitted on a patient two months after conception. In all animals used for these tests the reaction was negative. Three tests were performed on a third patient at intervals of two, two and a half, and three months after conception. Although different animals from different stocks were used for each test, all frogs gave negative responses. In a fourth case, two tests were performed at intervals of two, and two and a half months after conception. In both instances the findings were negative.

The cases just cited comprised 57 per cent of the "adjusted total errors." It would seem logical that these errors could have been caused, 1) by a lack of, or an insufficient amount of CGH in the urinary concentrate, or 2) by an increased threshold in the animals. No information was available concerning the prevalence of cases in which CGH was absent or at a very low level during the course of an otherwise normal cyesis. An occurrence of this sort would tend to decrease the accuracy of any pregnancy test which relies upon this CGH for its result. It would also account, in part, for some of the errors observed.

It has been shown that *R. pipiens* under different environments will react to varying minimal doses of CGH (8). In this laboratory, under conditions listed previously, 100 per cent of the frogs reacted to 40 I.U. of CGH. At the same time, 50 per cent of these frogs would react to 20 I.U., and 25 per cent to 10 I.U. The great variance in the degree of sensitivity to CGH among animals kept under similar conditions becomes readily apparent from the foregoing data. This is another factor which might affect the number of errors obtained.

Other explanations centered mostly upon errors in technique, such as inaccurate reading, leakage of the injected material, and inadequate extraction of hormone from the urine. The probability that these errors, if present, would be repeated in the cases in which more than one test was run appeared to be extremely low.

SUMMARY

Results of pregnancy tests are presented in which *R. pipiens* was used as the test organism. Under the experimental conditions stated, this frog may be used for testing as many as five times. An accuracy of 96 per cent was obtained. Possible explanations for errors are discussed.

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OBSERVATIONS ON HYPOPARATHYROIDISM. I. EXFOLIATIVE DERMATITIS AS THE PRESENTING SIGN OF HYPOPARATHYROIDISM: A CASE REPORT

A. HARELL-STEINBERG, M.D. (Lt. Col.), L. ZIPRKOWSKI, M.D. (Lt. Col.), S. HAIM, M.D. (Lieut.), J. GAFNI, M.D. (Maj.) and M. LEVIN, M.D.

The Endocrinological Unit and the Dermatological Department of the Government Hospital, Tel-Hashomer, Israel

IDIOPATHIC hypoparathyroidism has been known as a clinical entity for almost a generation; it is, however, surprising how few cases of this disease have been reported in the clinical literature (1–6). In most of these reports special emphasis is laid on the trophic ectodermal manifestation of the disease. Steinberg (6) in his review of the literature up to 1952 found that out of 52 cases recorded, 37 had some kind of trophic ectodermal change of varying character and severity. Nevertheless, there is only 1 publication (7) on the occurrence of exfoliative dermatitis as a manifestation of idiopathic hypoparathyroidism.[1] This fact has induced us to present the following case report.

CASE REPORT

Mrs. A.S., a 35-year-old Iraqi married woman, mother of 3 healthy sons, had been under treatment and observation since 1951 because of pulmonary tuberculosis. Psoriatic lesions had developed in 1952.

A month before admission to our hospital she was discharged from a tuberculosis sanatorium in a stable general condition, but a few days later returned to that hospital with an acute skin disorder. She was transferred to our dermatological department on February 9, 1953, with a diagnosis of acute toxic dermatitis due to the application of local medicaments.

She was found to be in an acute toxic state, disoriented, and suffering from attacks of dyspnea and cyanosis accompanied by respiratory stridor. Her temperature was 38.5° C. Her pulse was weak and over 120 beats per minute.

There was generalized exfoliation of the skin, which was erythematous, swollen and wet (Figs. 1 and 2). The hair was thin, dry and very sparse (Fig. 3). The mucous mem-

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[1] We were informed by Dr. C. E. Dent in a personal communication that he also observed exfoliative dermatitis in a patient with severe hypocalcemia following removal of a parathyroid tumor. He had another patient with postoperative tetany who many times suffered from eczema when the level of plasma calcium was low; the eczema improved every time the plasma calcium level rose.

FIGS. 1–3. The patient before treatment with vitamin D_2.

FIGS. 4–5. The patient during treatment with vitamin D_2, approximately five weeks after onset of therapy.

FIGS. 6–8. The patient on discharge from the hospital, receiving a daily maintenance dose of 50,000 units of vitamin D_2.

brane of the mouth and upper respiratory tract manifested a diffuse erythematous swelling. There was a pronounced leukocytosis of 52,000 per cu. mm. of blood, with a marked shift to the left and an increased number of eosinophils (6–10 per cent). The sedimentation rate was 105/123 (Westergren method). The serum protein concentration was low (5.64 Gm. per 100 ml.) and the albumin globulin ratio was reversed (2.71/2.93).

A roentgenogram of the chest showed a fibrosed tubercular process in the left apex.

The electrocardiographic findings aroused the suspicion of hypocalcemia, because of a prolonged QT. The serum calcium level proved to be 3.8 mg. per 100 ml. Chvostek's sign was present. The serum levels of the other electrolytes were within normal limits.

Faced with these findings we made further inquiries into the past history of the patient. She had not undergone thyroidectomy. However, she stated that since 1942, after an emotional and nervous strain, she started to suffer from attacks of fainting with shortness of breath accompanied by muscular twitching.

In spite of repeated physical examinations we were unable to elicit any additional signs or symptoms pointing to hypocalcemia, except for marginal cataracts. Teeth and nails were normal. Roentgenograms of the bones were noncontributory; there were no calcifications of the basal ganglia.

After admission the patient received the usual conservative treatment for generalized exfoliative dermatitis, but without improvement. The temperature became hectic and the pulse reached 140 beats per minute. Because of the serious condition of the skin and in spite of the tuberculous process, corticotropin (ACTH) therapy was begun, combined with a cover of streptomycin. The condition of the skin became worse, and tetany became more overt. ACTH was therefore stopped and only the treatment of hypocalcemia was continued.

The patient received initially 1,200,000 units of vitamin D_2 by injection within ten days. Dihydrotachysterol (AT-10) was added on the eleventh day (0.625 mg. three times daily), and after two weeks was given alone. At the beginning of treatment with vitamin D_2 the level of calcium in the peripheral blood was 3.8 mg. per 100 ml. After eleven days, at the beginning of the combined treatment, the calcium level rose to 4.5 mg. per 100 ml.; thirteen days later it reached 7.1 mg.; and at the end of forty days of treatment it had risen to to 10.45 mg. At the same time there was an almost parallel improvement in the general condition of the patient. Fifteen days after the first injection of vitamin D_2 and four days after the onset of treatment with AT-10, the skin and the patient's general condition started to improve. The dyspneal attacks cleared up. After three weeks the patient was able to sit in an easy chair, and at the end of five weeks she could walk (Figs. 4 and 5). The dosage of AT-10 was gradually reduced and then the drug was discontinued.

At this point there was additional evidence that her skin condition was intimately connected with the values of calcium in the peripheral blood. Sixteen days after stopping AT-10, the serum calcium level fell to 6.9 mg. per 100 ml., and the skin once more became swollen and erythematous. AT-10 was again given and within ten days' time the serum calcium level rose to 9.1 mg. per 100 ml. and the skin reverted to normal. The patient was discharged on June 18, 1953, in good condition (Figs. 6, 7 and 8), receiving a daily maintenance dose of 50,000 units of vitamin D_2—a dose which had proved to be adequate during the last three weeks of hospitalization.

DISCUSSION

The presenting symptom in this patient was exfoliative dermatitis.

The condition could possibly be attributed to the following factors, although the first three are extremely unlikely:

1. *Tuberculosis*

Exfoliative dermatitis occurs usually in the acute stages of pulmonary tuberculosis; in our patient the process was quiescent.

2. *Psoriasis*

In psoriasis, exfoliative dermatitis appears mostly in cases in which the disease is generalized; in our case there were only minor psoriatic lesions limited to minute areas of the skin (8).

3. *Local treatment*

It is more difficult to exclude the influence of local treatment. (The patient received before admission to the hospital different kinds of unguenta, the value of which is difficult to trace. The last one was Cremor Ichthyolimitis cum Ac. Salicyl).

ACTH usually leads to dramatic improvement in such cases. We can deduce that in our patient previous local treatment with ACTH was not the cause of the exfoliation, as our use of ACTH had no effect on the skin condition. This fact confirms also our doubts that tuberculosis and psoriasis were decisive factors in producing the dermatitis, as in both conditions ACTH should have influenced the skin lesions beneficially (9).

4. *Hypocalcemia*

The situation is different with regard to the hypocalcemia. Results of the therapeutic tests were very suggestive. As soon as the serum calcium level rose, the skin condition improved; and conversely, as soon as the calcium level fell the lesions appeared again—leading to the reasonable assumption that the hypocalcemia played a decisive role in producing the exfoliation. It is a matter for speculation whether hypocalcemia was the only cause or whether it was merely a contributing factor. We tend to believe that it was a contributing factor producing exfoliative dermatitis in a patient suffering from psoriasis. This assumption is strengthened by the fact that the only other case of exfoliative dermatitis observed in hypoparathyroidism also occurred in a patient suffering from psoriasis (Lachman (7)).

SUMMARY

A case of hypoparathyroidism with a good response to AT-10 and vitamin D_2 therapy in a 35-year-old woman has been described.

The presenting sign of the disease was generalized exfoliative dermatitis which healed perfectly during treatment of the underlying hypocalcemia.

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OBSERVATIONS ON HYPOPARATHYROIDISM. II. INACTIVATION OF PARATHYROID HORMONE IN A CASE OF CLINICAL HYPOPARATHYROIDISM

A. HARELL-STEINBERG, M.D. (Lt. Col.), L. ZIPRKOWSKI, M.D. (Lt. Col.), S. HAIM, M.D. (Lieut.), J. GAFNI, M.D. (Maj.) and M. LEVIN, M.D.

The Endocrinological Unit and the Dermatological Department of the Government Hospital, Tel-Hashomer, Israel

IN THE preceding article (1) we have described a case of generalized exfoliative dermatitis associated with hypoparathyroidism.

This paper is concerned with biochemical investigations on the same patient.

It is customary to distinguish two forms of clinical hypoparathyroidism —true idiopathic hypoparathyroidism and pseudohypoparathyroidism. The latter condition has been described by Albright *et al.* (2). Clinically it is indistinguishable from true idiopathic or postoperative hypoparathyroidism. In the pseudo form, however, the defect seems to originate not in the parathyroid gland itself but in the target cells (*e.g.*, kidney, bones), which are refractory to the parathyroid hormone (3). The defect appears to be congenital and leads to disturbances in development predominantly affecting the skeleton. The patients are short. Their metacarpal and metatarsal bones are also short, but in an irregular manner, so that often there are marked differences in length of fingers and toes. The patients usually have a round face, a short neck, calcifications in the basal ganglia, cataracts, strabismus, and other congenital malformations.

Our patient, although having an initial serum calcium level of 3.8 mg. per 100 ml., did not exhibit any morphologic peculiarities (1). There were no bone deformities, no dwarfism, no shortening of the metacarpal or metatarsal bones and no calcification of basal ganglia. A rounded face and pyknic build are so often encountered in the population that it could not be described as pertinent for the diagnosis.

Ellsworth and Howard described a method for testing the responsiveness of the target cells (4). In persons with physiologically active parathyroids an injection of parathyroid hormone increases phosphorus excretion. Patients with pseudohypoparathyroidism, however, are unable to respond in

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this fashion to parathyroid hormone and their phosphorus excretion remains unaltered after the injection.

DATA AND DISCUSSION

When our patient was subjected three times to the Ellsworth-Howard test (200 units of Parathormone intravenously), the results were as follows: During the first test the mean value of phosphorus excretion rose from 14.2 mg. to 25.2 mg. per hour, during the second test from 13.2 mg. to 20.2 mg. per hour, and during the third test only from 38.5 mg. to 46.0 mg. per hour (Table 1).

TABLE 1. RESULTS OF ELLSWORTH-HOWARD TESTS IN PATIENT A.S. (COLUMNS 1–3) COMPARED WITH RESULTS IN A PATIENT SUFFERING FROM POSTOPERATIVE HYPOPARATHYROIDISM (COLUMN 4) AND IN A CASE OF IDIOPATHIC HYPOPARATHYROIDISM (COLUMN 5)

	Phosphorus excretion (mg./hr.)				
	In Patient A.S.			In a case of postop. hyparathy'm	In a case of idiopathic hypoparathy'm
	First test	Second test	Third test		
4 hrs. before injection of Parathormone	—	19.4	—	—	—
3 hrs. before injection of Parathormone	—	10.3	—	—	—
2 hrs. before injection of Parathormone	14.0	10.9	45.1	13.8	9.3
1 hr. before injection of Parathormone	14.4	12.3	32.0	12.2	9.8
Mean	*14.2*	*13.2*	*38.5*	*13.0*	*9.5*
1 hr. after injection of Parathormone	28.0	23.9	46.2	58.0	48.0
2 hrs. after injection of Parathormone	25.0	23.8	54.4	56.9	25.2
3 hrs. after injection of Parathormone	23.8	21.1	37.6	—	25.3
4 hrs. after injection of Parathormone	24.0	12.2	—	—	—
Mean	*25.2*	*20.2*	*46.0*	*57.5*	*32.8*
Increase after injection of Parathormone	*77.5%*	*53%*	*19%*	*342%*	*245%*

As a control study, we performed the same test on 1 normal subject, on 1 patient with idiopathic hypoparathyroidism, and on 1 with postoperative hypoparathyroidism. The rise in urinary phosphorus in the normal subject was about 200 per cent. In the postoperative patient the rise was from 13.0 mg. to 57.5 mg. per hour and in the patient with idiopathic hypoparathyroidism it was from 9.5 mg. to 32.8 mg. per hour (Table 1).

The only evidence in favor of pseudohypoparathyroidism in our patient was the results of the Ellsworth-Howard test on two occasions following an equivocal result on the first trial. This raises several questions:

1. *Was the parathyroid preparation active?*

We were able to prove that the parathyroid hormone preparation (Parathormone) was active, as there was a rise of 342 per cent in phosphorus

excretion after the injection of 200 units of Parathormone in the patient with postoperative hypoparathyroidism (Table 1).

2. *Is the biochemical evidence of end-organ unresponsiveness conclusive?*

There are certain aspects of the results which reduce the significance of the tests and make their finality questionable.

A. Prunty (5) called attention to the fact that some of hypoparathyroid patients in whom the disease develops after thyroidectomy respond less readily to parathyroid hormone than do patients with similar case histories. Before operation the end-organs of these patients had been reacting perfectly well.

Based on this fact and on their own experience, Robinson, Carmichael and Cumings (6) reached the conclusion that results of the Ellsworth-Howard test may be misleading. Similar opinions are held by Stewart and Bowen (7) and Dent (8).

B. All workers in this field are aware that patients who receive dihydrotachysterol (AT-10) or calciferol (vitamin D_2) respond poorly to Parathormone. This could explain the results in test 3. The high excretion of phosphorus was most probably due to AT-10 therapy which inhibited the effect of Parathormone (Table 1).

If the target cells were refractory to parathyroid hormone, then undoubtedly this physiologic defect would have provoked changes in the skeleton such as were classically described by Albright and others (1). The lack of bone deformities was actually proof that at least during the years of maturation the target cells reacted well to parathyroid hormone.

3. *Is there a third form of hypoparathyroidism?*

For the foregoing reasons we assumed that possibly there is a third, acquired form of hypoparathyroidism in which neither the gland itself nor the target cells are pathologically involved and in which the pathologic disturbance is to be found elsewhere. The suggestion is that substances in the blood of the patient can destroy the parathyroid hormone.

In order to examine this possibility the following tests were performed (Table 2): A dose of 200 units of Parathormone was incubated for twenty-four hours at 37° C. and the conventional Ellsworth-Howard test carried out on a healthy person; there was an increase of 72 per cent in phosphorus excretion. Then the test was performed slightly differently: The dose of 200 units of Parathormone was incubated for twenty-four hours in 10 cc. of physiologic saline, and a similar dose in 10 cc. of serum from a healthy person. After the intravenous injection of these solutions into healthy subjects there were rises in phosphorus excretion of 196 per cent and of 102 per cent, respectively. The results were the same as with the conventional Ellsworth-Howard test.

When, however, the parathyroid hormone was incubated in the serum of Patient A.S. and then injected into a healthy person there was no rise in phosphorus excretion. The mean hourly excretion before injection on the first occasion was 22.3 mg., per hour and after injection, 21.75 mg.; on the second trial it was 21.4 mg. before and 22.7 mg. after injection; and on the third trial it was 37.2 mg. before and 39.9 mg. after injection.

However, inasmuch as there were variations in phosphorus excretion (Table 2), we were reluctant to draw definite conclusions. The results are

TABLE 2. RESULTS OF ELLSWORTH-HOWARD TESTS IN HEALTHY SUBJECTS, AFTER INCUBATION OF PARATHORMONE IN VARIOUS MEDIA, INCLUDING SERA OF HYPOPARATHYROID PATIENTS

	Phosphorus excretion (mg./hr.)						
	Parathormone incubated at 37° C. for 24 hrs.			Parathormone incubated at 37° C. for 24 hrs.			
	Alone	In saline	In serum of a healthy person	In serum of Patient A.S.			In serum of patient refractory to parathyroid hormone
				First test	Second test	Third test	
4 hrs. before injection of Parathormone	—	—	—	36.4	—	—	—
3 hrs. before injection of Parathormone	—	—	—	22.8	27.9	60.5	—
2 hrs. before injection of Parathormone	57.10	9.75	31.00	6.1	19.2	22.5	20.0
1 hr. before injection of Parathormone	51.50	5.20	12.70	26.1	17.1	28.6	23.4
Mean	*54.30*	*7.47*	*21.85*	*22.3*	*21.4*	*37.2*	*21.7*
1 hr. after injection of Parathormone	94.50	23.60	34.80	7.44	13.4	50.0	24.2
2 hrs. after injection of Parathormone	93.00	22.30	41.30	21.30	28.0	34.2	8.6*
3 hrs. after injection of Parathormone	—	20.50	56.40	36.50	26.7	35.6	35.1
4 hrs. after injection of Parathormone	—	—	—	—	—	—	13.3
Mean	*93.75*	*22.10*	*44.10*	*21.75*	*22.7*	*39.9*	*20.3*
Increase after injection of Parathormone	*72%*	*196%*	*102%*	*0%*	*0%*	*0%*	*0%*

* This collection was incomplete and part of the urine was added to the collection of the next hour.

nevertheless too striking to be ignored and in our opinion justify an additional study to prove whether there exists a third form of hypoparathyroidism. It might be especially rewarding to re-examine patients who exhibit no skeletal malformations but do not respond to intravenous injection of parathyroid hormone.

We are strengthened in our suggestion by the following fact: A patient (H.Z.) who for twelve days received injections of Parathormone became resistant to this hormone. When a dose of 200 units of Parathormone was incubated in 10 cc. of serum from this patient, there was no rise of phosphorus excretion following the intravenous injection of this solution into a

healthy subject. The mean hourly excretion was 21.7 mg. before and 20.3 mg. per hour after the injection (Table 2). The results were similar to those obtained in Patient A.S. and seem to support the assumption that some substances may occur in the blood which destroy the parathyroid hormone.

The character of these substances has not yet been determined. It is even possible that in Patient A.S., repeated testing with parathyroid hormone led to production of antihormone (Table 3). However, the disease started years before the parathyroid hormone was used. It is, therefore, more likely that these substances were initially present in the patient's serum.

TABLE 3. AMOUNT OF PARATHYROID HORMONE RECEIVED BY PATIENT A.S. COMPARED WITH THAT RECEIVED BY PATIENT H.Z. WHO BECAME RESISTANT TO THIS HORMONE

Date	Patient A.S.		Patient H.Z.		Date
Oct. 12, 1953	First Ellsworth-Howard test	Parathormone, 200 units i.v.	Each day for 12 consecutive days		Dec. 23, 1954
Oct. 27, 1953	Second Ellsworth-Howard test	200 units i.v.		100 units i.v.	Jan. 4, 1955
Dec. 23, 1953	Third Ellsworth-Howard test	200 units i.v.			
	Total amount 600 units i.v.		*Total amount 1200 units i.v.*		

SUMMARY

A case of hypocalcemia is presented in which the usual clinical and biochemical features of either true idiopathic hypoparathyroidism or pseudohypoparathyroidism were incomplete.

Evidence was obtained in keeping with the supposition that substances circulating in the patient's serum inactivated the parathyroid hormone, thus raising the possibility of the existence of a third variety of hypoparathyroidism.

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CALCIUM METABOLISM, BONES AND CALCIUM HOMEOSTASIS. A REVIEW OF CERTAIN CURRENT CONCEPTS*†

JOHN EAGER HOWARD, M.D.‡

The Department of Medicine, The Johns Hopkins Hospital, Baltimore, Maryland

THE purpose of this paper is to discuss some current views on the physiology of bone and the factors which control the metabolism and economy of calcium in the body as a whole. There are tremendous gaps in our knowledge. Nevertheless, this is an opportunity to review our thoughts and attitudes on these matters as they have developed over the years.

In the field of modern medical investigation the clinician is usually placed at the bottom of the totem pole, beneath his colleagues versed in the more basic sciences. Yet for a chemical or physical thesis to be accepted as physiologically sound for the human being, it must be shown to operate in *him*, sick or well. In this respect the clinician runs the final laboratory experiment for his colleagues; and, in addition, from his observation seat, often can provide clues of import to medical advancement. Furthermore, his opportunities for viewing the subject in its broadest aspects are apt to be greater than those of the biochemist or physicist. In the discussion to follow, I shall try to portray the bones as living cellular units, holding within their confines vast stores of mineral, especially calcium. As the result of forces operating upon the skeletal units, bones have the capacity to grow, repair themselves and break down; but in addition it is through the mediation of their powers to alter the flow of calcium into or out of their confines that the calcium concentration of our internal milieu is so closely guarded.

THE SKELETON IN THE HOMEOSTASIS OF CALCIUM

Metabolism

We shall begin with a review of certain basic facts, founded upon the simplest sorts of data and deductions. When one deprives the normal animal of calcium, the procedure can be withstood for long terms without an appreciable fall in the calcium concentration of the extracellular fluids; in

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‡ Associate Professor of Medicine, The Johns Hopkins University School of Medicine.

rats this can occur for several generations, with the only visible result a marked thinning of the skeleton (1). Situations that are stressful upon the calcium economy (such as prolonged lactation (2), steatorrhea (3) and even acutely repeated withdrawals by bleeding and retransfusion with calcium-free blood so that more calcium is removed than had been present in the entire extracellular compartment) are followed by prompt restitution of serum calcium concentration (4). Studies of the ash content of normal man indicate that, of some 1,200 grams of calcium present, all but 12 grams (99 per cent) is resident in the skeleton (5). From the low exchange rate with isotopic calcium (6), it appears certain that *intracellular* calcium is mostly "bound" and not in readily available form. There is about ½ gram in the entire *extracellular* compartment (7). Thus from purely quantitative aspects, any large or long-term deficit of calcium must be met from the skeleton, if serum calcium concentration is to be maintained. Data of similar type indicate that the bones are the site of uptake during circumstances of calcium plethora, though under these circumstances the kidneys render great assistance by excreting large moieties of the excess calcium (8). A low serum calcium level results in tetany, convulsions and dementia, whereas a high level causes serious damage to the kidneys (9). For its part in the homeostasis of calcium, our skeleton is therefore indispensable to life.

Lest some gastro-intestinal or renal physiologist rise in wrath against me, let me quickly say that these two systems also play important roles in calcium economy. In chronic losses from these areas, as in steatorrhea (3) or acidosis (10), serious calcium depletion can occur. Under normal circumstances, however, there is little more than 100 mg. of calcium absorbed per day from the gut (7) and there is no positive excretory mechanism (to our knowledge) provided for calcium elimination by the gastro-intestinal tract. In even severe, damaging hypercalcemias the urine rarely contains more than 500 mg. of calcium in twenty-four hours (7). *In the adjustment of major derangements of calcium homeostasis, the kidneys and the intestinal tract are of minor importance in comparison with the bones* (7).

Structure

Let us turn now for a moment to the structure of the skeleton in an effort to discern what manner of tissue this is which serves so important a chemical function.

The osteone (for lamellar or "hard" bone) and the trabecula (for the soft or cancellous type) may be visualized as the structural units of bone. Within the former is a minute artery and a vein (Fig. 1). The trabeculae are completely surrounded by extracellular fluid of the highly vascular marrow (Fig. 2). In both types, throughout the tissues, there are spaces (canaliculi and lacunae) through which tissue fluids presumably flow. Bone cells, or

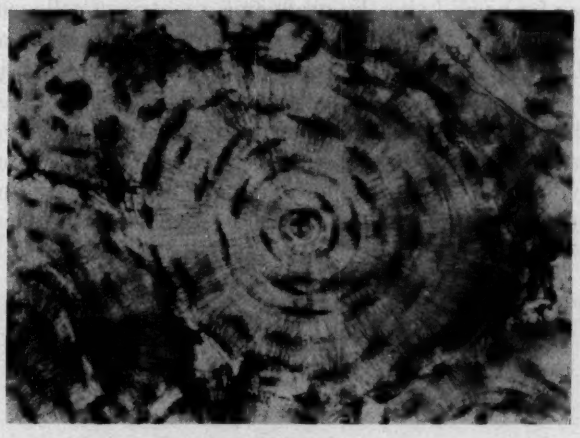

FIG. 1

FIG. 2

FIG. 1. Transverse section of cortical human bone showing several osteones with central vascular channels. (Reproduced from Bone and Bones; Fundamentals of Bone Biology. St. Louis, C. V. Mosby Co., Weinmann, J. P., and Sicher, H.: 1955, p. 22, through the courtesy of the authors and permission of the publisher.)

FIG. 2. Trabeculae surrounded by normal marrow showing physiologic osteoid (a) in a premature infant. (Courtesy of Dr. E. A. Park.)

osteoblasts, are members of the fibroblastic clan, differing from ordinary fibroblasts, so far as we know, by producing a matrix of collagen and polysaccharide, along which are deposited calcium salts when the environment is normal. These apatite salts are relatively constant in composition, being formed of what the physical chemist would anticipate from the qualities of the extracellular fluids, and having also other molecules such as sodium, potassium and citrate[1] at crystal surface points (11, 12). The crystals are smaller in younger or newer bone, and such bone contains more water per unit of mass (13). The crystals are arranged at specific spacings along the collagen fibrils, in highly organized fashion (Fig. 3).

FIG. 3. "A section of periosteal bone from a 16-day embryo, showing the periodic structure of the collagen fibrils; apatite particles are localized in one interband of each period, namely, between the *d* and *ab* bands. ×91,000." (Reproduced from Jackson, S. F., and Randall, J. T.: The Fine Structure of Bone, *Nature* **178**: 798, 1956, through the courtesy of the authors and with permission of the publisher.)

In *growth of bone* one should visualize a newly formed matrix added to the surface just under the layer of osteoblasts. When this calcifies, a new layer of matrix is laid upon the older, pushing it back—farther from the site of most active exchange with the extracellular fluids, but still a storehouse that could become available if the surface areas should become unclothed and expose it. It has been estimated by expert crystallographers that there are two to four acres of surface area in the skeleton where exchange might readily take place between bone salt and extracellular fluids (14). This is an ideal arrangement for the mechanics of a buffering or homeostatic set-up, as described.

Bone is a tissue very much alive, being constantly destroyed and newly formed (15). These processes occur over and above the purely chemical exchange reactions between atoms of the crystals and the extracellular fluids, so beautifully demonstrable with radioactive isotopes. The histologist sees

[1] W. F. Neuman (personal communication) has found repeatedly that citrate levels in bone are 8 or 10 mg. per 100 ml. as compared to 4 mg. per 100 ml. in serum.

both areas of destruction and of new bone formation. (Osteoid is the name given to the matrix as yet unclothed by crystals.) The two processes may occur adjacently in a single trabecula, and one or the other may predominate in a given general area (Fig. 4). Significant is the fact that one rarely, if ever, sees in the destructive process, matrix from which the crystals have been removed; both seem to be resorbed together (16). Yet, in rapidly growing new bone, as around fractures or when the environment is not

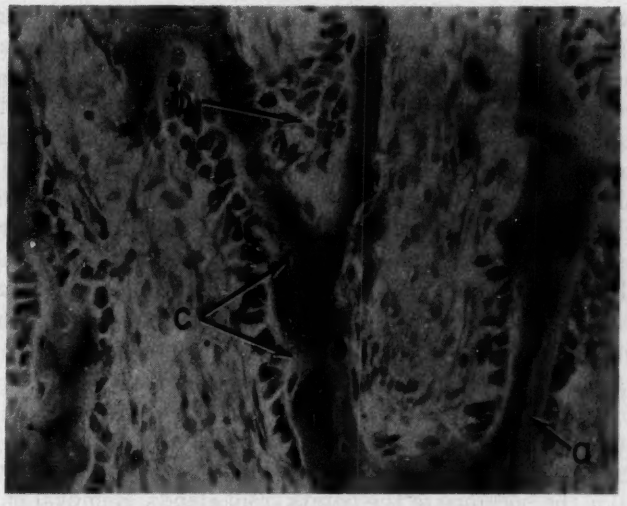

FIG. 4. Rib from a premature infant showing characteristic intense formation of new bone. The osteoblasts are not only numerous but large, and one can see osteoid coverings (**a**) of the calcified bone which they have formed. Where the section happens to be tangential, these cells form a carpet (**b**). In close proximity to these areas of osteoblastic proliferation are visible areas of destruction (**c**), in which the endosteal membrane breaks up and osteoblasts form through coalescence of osteoblasts. (Courtesy of Dr. E. A. Park.)

suitable for crystal deposit as in rickets, osteoid is readily visible as a distinct wide border (16), *always within a layer of osteoblasts.*

It is hard to doubt that bone cells can in some way respond to purely physical stimuli. The local atrophy of disuse, which occurs when a member is put in a cast or splint or has its nerve supply severed, takes place to an astonishing extent and with great rapidity, as can be seen if one compares its x-ray picture with that of the opposite member (17). Contrariwise, use of a part results in hypertrophy of the bone as well as of the soft tissues of the area (18). Whether these alterations are induced by changes in capillary flow, CO_2 pressure or what-not, is unknown; but in picturing skeletal econ-

omy, the important role that physical factors can play must not be overlooked.

Why then does a bone not atrophy to the point of disappearance or loss of all its structure and form? There seems to be a sort of inherent plan, perhaps resulting from the same type of force which makes a few properly selected cells of the embryo continue to grow and form a previously designated structure such as femur or liver, even though removed to a totally different area. Upon such a basic plan secondary forces such as stress, strain or abnormal nutrition can play, resulting in either hypertrophy or atrophy. This inherent force toward maintenance of design (termed an "organizer" by the embryologists) may explain why, even during a state of tremendous atrophy or destruction by an excess of parathyroid hormone, there will be evidence of new bone being laid down along individual trabeculae at various points.

These brief outlines of concepts relative to skeletal structure will be needed when we shall later attempt to theorize regarding just what forces play upon the skeleton to maintain calcium homeostasis in the normal subject, how aberrations from the normal environment may affect the skeleton, and how abnormalities of the skeleton may upset the body's chemical environment.

THE PARATHYROIDS

The most potent, or at least the most rapidly acting force in affecting the concentration of calcium in the body fluids (and hence, in playing upon the bones) is the hormone of the parathyroid glands. Removal of the parathyroids is the only certain and reasonably physiologic method of rapidly lowering the level of serum calcium (7). This may occur within a few hours of total parathyroidectomy and is not accompanied, so far as we can discern, by any loss of calcium to the external environment; hence the small amount disappearing has gone to the skeleton. The *calcio*stat is now changed to a lower setting, just as one controls the automatic *thermo*stat. As everyone knows who has treated parathyroid tetany, if one introduces calcium salts into the blood, the level rises only so long as injection continues, falling back promptly to the starting level. Even more interesting is the fact that removal of calcium from the parathyroidectomized animal, as by bleeding and replacement with calcium-free blood, is also quickly followed by restoration of calcium level, *but only back to the starting concentration* (4b). Thus there is a fundamental or basic mechanism of calcium homeostasis, upon which the parathyroid hormone acts. Like other glandular secretions the parathyroid hormone does not initiate a biologic process but only plays upon it, by speeding or slowing the activity over a fundamental pathway.

We now know that the parathyroid hormone influences bone directly. There was once a violent polemic in the medical literature which revolved about the question whether Parathormone affected bone only by way of its indirect action on the movement of phosphorus (19). This argument was settled by two pieces of information: 1) in animals given the hormone, hypercalcemia occurred and osteitis fibrosa developed even when the kidneys were removed (20, 21) *i.e.*, when the serum phosphorus level was steadily rising; and 2) that bits of parathyroid tissue placed subperiosteally produced local osteitis fibrosa (22, 23). However, it cannot be doubted that *movement of phosphorus* is also an accompaniment of the action of parathyroid hormone, as phosphate diuresis is the first detectable effect of administration (24). This is most dramatically seen in the parathyroidectomized organism, in which a twenty- to fifty-fold increase in urinary phosphorus may be demonstrated within an hour (25); and also in the patient with a functioning parathyroid adenoma, in whom the serum phosphorus concentration is usually low (26).

Therefore, it may be stated without fear of contradiction that the parathyroids exert activity in three spheres—upon calcium metabolism, upon bone cells and upon phosphorus metabolism. Whether these three actions be all from the same material acting at different sites, or from three separate chemical substances excreted by the glands, is another matter. The present commercially available product of extraction is an extremely crude and impure one. When better methods of assay are available, the true nature of parathyroid secretion may be discovered, followed inevitably by a rapid advance in our knowledge of calcium metabolism.

Before leaving the parathyroids, it would be well to discuss briefly our concept of their role in calcium homeostasis. Though hyperplasia of a gland does not prove its hyperfunction, it nevertheless arouses suspicion thereof. Since the parathyroids of animals with low-calcium rickets uniformly are enlarged and those of animals with low-phosphorus rickets are not (27), it had been deduced that a low level of serum calcium was a stimulus to increased parathyroid activity. This deduction was fortified by perfusion experiments wherein an infusion of low calcium concentration resulted in a perfusate from the thyroid-parathyroid veins containing an increased amount of material with parathyroid activity (28). When normal man is given an intravenous injection of calcium salts and the serum calcium concentration is thus increased, there is characteristically a lowered phosphorus output by the kidney despite a rising blood phosphorus concentration (8)—a type of response typical of parathyroid deficiency (24). One may therefore reasonably conclude that the parathyroids are sensitive to changes in serum calcium concentration, responding quickly to a fall by increasing their output of hormone, and to a rise by reducing their hor-

monal output. Since increased parathyroid secretion causes a rise in the level of serum calcium, and removal of the parathyroids causes a fall in the serum level, such an endocrine system offers a superb stabilizing mechanism in maintaining calcium concentration within narrow limits. The site of operation of this system must be at the skeletal level, where the surfaces of the structural units—trabeculae and osteones—meet extracellular fluids.

VITAMIN D

It is our belief that vitamin D, also has an action at the level of the skeletal units, in addition to its known action of increasing calcium absorption from the gut and a probable action on renal phosphate reabsorption. Thus, when one sees steatorrhea in sun-bathed Puerto Rico, there is never hypocalcemia or tetany; yet in 50 per cent of the Baltimore cases these phenomena are present and hypocalcemia is almost characteristic of the disease as seen in Boston or Montreal (29, 30). When one starves the rat undergoing experimental low-phosphorus rickets, there is a sharp fall in the level of serum calcium. This does not occur, however, when the normal rat is starved (16), nor in the rat given a low-phosphorus diet with added vitamin D (16). Furthermore, when a rat becomes poisoned with large doses of vitamin D, a large calcium deficit ensues (31), with the appearance of enormous osteoid borders along the trabeculae (32). The reason for the marked difference in effect of small and huge doses of vitamin D remains to be determined.

TRANSPORT OF CALCIUM AND PHOSPHORUS

Though the circulating blood carries nutriment to, and waste products away from the sites of active utilization, it is only those portions of the blood coming in contact with cells which truly concerns us—that portion of the calcium and phosphorus which traverses the capillary walls and reaches the cell in a biologically usable form. When members of the "bone club" speak of serum or urinary phosphorus they mean inorganic phosphate. The transport of this compound offers little problem; so far as is known, nearly all so-called acid-soluble phosphorus in serum and urine is in this form and is freely diffusible through ordinary semipermeable membranes. Thus the inorganic phosphorus of the plasma or serum is the same as that of the extracellular fluids, and that of the glomerular filtrate is the same as that of the serum, at least under all but the most unusual circumstances (33). However, a considerable portion of the serum calcium is bound to the proteins, and hence will not pass in any quantity through normal capillary membrane. A very small portion is chelated by citrate and other chelating materials of the serum, and bioassay shows that this

also is not available to, or biologically useful to the cell (34, 35). We have no direct way of measuring the "useful" calcium in extracellular fluid. By indirect means, the following estimations have been made of the proportion of total serum calcium that can be utilized by the cell: by the frog-heart method, about half (34); by ultrafiltration studies in our laboratory, 65 to 70 per cent (33); and by new ultrafiltration techniques and physico-chemical calculations, 60 per cent (36). The latter value agrees well with that obtained when sera are tested against preparations of rachitic cartilage (37). Whatever may be the correct proportion, it is important to remember that it is the ionized fraction of the calcium in extracellular fluid that is available to the tissues, and hence of biologic interest; the portion which may be protein-bound or citrate-chelated apparently is not involved in the *bio*chemical picture. This may sound extremely complicated, but practically this is not the case for the clinician. The albumin of serum binds the greater portion of calcium (38), and hyperalbuminemia is virtually not met unless artificially induced. Thus an elevated serum calcium level (usually measured as total calcium) means a high level of calcium in the interstitial fluids, and hence something awry with the usual homeostatic controls. On the other hand, a low level of total serum calcium can be accounted for by the common clinical finding of hypoalbuminemia. In this instance, the concentration of calcium in the extracellular fluid may be normal, and there may be nothing amiss with calcium homeostasis (38). In order not to confuse the picture unnecessarily, let us assume in the discussion to follow that the levels of serum proteins are normal, so that hypercalcemia will mean a high concentration of calcium in the interstitial fluid and a low serum calcium level will mean a low concentration in the interstitial fluid.

Saturation and solubility products. In order to visualize the state of calcium and phosphorus in the body fluids, we must come to some sort of terms with the chemist and refer to such items as saturation and solubility products. Much thought has been given recently to these matters and Neuman has reviewed the evidence, concluding that serum is supersaturated with calcium and phosphorus as compared to bone (11). We know from both *in vitro* and *in vivo* observations that calcium and phosphorus can be added to serum in modest quantities without immediate precipitation taking place (33). To cite a ready analogy, table salt may be dissolved in water in quantities considerably higher than the chemical saturation point and the solution remain clear. However, drop so little as a single crystal of sodium chloride into this solution and a cloud develops, followed by crystalline precipitation. A seeding principle seems at present the most likely mechanism for the formation of the crystalline salts of the bones. When a salt of slightly different character but similar in size and electrical configuration incites the deposition of crystals, the reaction is known as epi-

taxis. Neuman visualizes the bone matrix as having in or on its surface such an epitactic agent to initiate deposition of apatite crystals from supersaturated extracellular fluid (11)—this being bone matrix' point of differentiation from the collagen of the ordinary fibroblast. At the moment this is hypothesis, or even pure speculation, but in the light of current knowledge it is a shrewd and plausible one.

On rachitic cartilage, *in vitro* studies have yielded much information regarding biologic calcification. In the early days of such investigation, Howland and Kramer observed that when the Ca×P product of an infant's serum was below a certain value, rickets was present; when it was above this value, rickets was either healing or absent (39). Furthermore, they noted that, *in vitro*, uncalcified rachitic cartilage would become calcified in serum with a Ca×P product above the threshold, but would not become calcified when the Ca×P product of the serum (or an artificial solution) was below this level. Using serum ultra-filtrates and making allowance for the fact that the concentration of serum phosphorus is normally higher in the rat than in the human subject, Howland and Kramer's rule has been found to hold under a wide variety of circumstances (37). The only known exceptions are in the rare condition known as hypophosphatasia (40) and in renal insufficiency (37). In the latter, one often finds juvenile-type rachitic uncalcified cartilage in the presence of a nearly normal level of serum calcium and a high level of serum phosphorus (41); moreover, serum from uremic patients with such elevated Ca×P products often fails to calcify rachitic cartilage *in vitro* (37). There appears to be something in the serum of uremic patients (not elevated concentrations of potassium, magnesium or urea, so far as is known) that prevents or hinders deposition of lime salt under conditions which would otherwise result in its deposit (37).

Before leaving the subject of calcium and phosphorus transport and Ca×P products—and Neuman insists there can be no solubility product estimated for the situation as it exists in biologic fluids—two other facts are perhaps worth remembering. When ordinary serum or its ultrafiltrate is agitated unstoppered at body or room temperature, a precipitate will form because of the change in pH from 7.4 to 8.0 resulting from escape of carbon dioxide; therefore, experiments dealing with *in vitro* calcification must be performed under conditions of controlled pH. As applied to the human body, this would mean that a local rise of pH would tend to precipitate lime salts from normal body fluids.

The other fact of clinical importance is that when the level of either calcium or phosphorus in serum is raised above a critical point, there is a change in the properties of these elements with regard to ultrafiltration, and relatively less of each passes the ultrafilter than expected (33). With a

normal phosphorus level the critical figure for calcium concentration is about 20 mg. per 100 cc.; with a normal calcium level the critical figure for phosphorus is 8–10 mg. per 100 cc. In man or the experimental animal, when serum calcium concentration exceeds the critical level—however induced—death rapidly ensues with shock, anuria and extraordinary dehydration of the blood (38).

THE KIDNEYS

The kidneys and the gut are relatively impotent in dealing with emergency problems of calcium homeostasis, but we should pay our highest encomiums to the kidney for its capacity to resorb the calcium filtered at the glomerulus. It is this renal action of resorbing normally 99 per cent of the filtered calcium which permits the serum calcium to exist at its usual supersaturated level. Without these conditions, according to our present concepts, there would be no bone mineral. We are ignorant, however, as to just how calcium and phosphorus are dealt with by the kidney and the intestine. In the normal healthy man on a diet say of 500 mg. of calcium per day in the form of milk, about 100 to 150 mg. of calcium will appear in the urine. In terms of overall movement, therefore, there has been a net gain of only 150 mg. of calcium absorbed from the intestinal tract (starting with the assumption that calcium balance is in equilibrium). Upon raising the intake to 2,000 mg. per day, the urinary calcium rises to only 250 mg. per day (7). Gamble has calculated that 8 liters of various digestive juices (from stomach, duodenum, pancreas and gallbladder) are poured into the tract per day (42). If these be represented as mostly serum ultrafiltrate, they would contain about 500 mg. of calcium. In times of calcium need, when no calcium is being ingested, the gut is clearly helpless to aid the situation. When calcium is available in the diet, the gut can, and sometimes does respond with greater absorption. Under the stimulus of growth, for example, more is absorbed and yet the same quantity is excreted in the urine (43a). Vitamin D also clearly increases intestinal absorption of calcium (43b). However, no positive excretory mechanism has been demonstrated whereby the gut might assist in losing calcium to aid in overcoming a plethora of calcium in the serum.

How calcium is handled by the kidney is unknown. The 24-hour urinary excretion of calcium is only about 100–150 mg. Even in cases of severe hypercalcemia, we have never seen more than 1 gram of calcium appear daily in the urine, and hypercalcemia per se is a very damaging agent to the kidneys. Yet, during excessive dosage with vitamin D, there is often a marked increase in urinary calcium even while the serum calcium level remains normal; and during treatment of hypoparathyroid patients with

vitamin D there is heavy calciuria, often with serum calcium concentration well below normal (44). The existence of a calcium-excreting mechanism, positive and not passive, is strongly suggested by such observations.

CELLULAR MECHANISMS OF BONE

In the system of calcium homeostasis which has been pictured, it seems that for several reasons there must be government by living cellular mechanisms. Though rachitic cartilage, produced in an environment of low Ca×P product, readily becomes calcified *in vitro*, no one has ever seen rachitic osteoid become calcified in the test tube. The immediacy with which, on removal of the parathyroids, the calciostat is lowered to a new unshakable level suggests a widespread change at the skeletal surfaces—the only place with the capacity for such a massive sudden change. The calcium salts of bone exist in crystalline state with their hydration layers. To effect their egress from bone into the supersaturated extracellular fluid, some local solubilizing mechanism must be involved, and this implies the activity of living tissue. Such a concept suggests a barrier with a gradient, which can be influenced by local (stress, trauma, blood supply), systemic and hormonal forces. Whether or not one visualizes this barrier as an endosteal membrane (45), as some experts have been unwilling to do (45), makes little difference, so long as it is viewed as being under the control of living matter—hence presumably enzymatic.

Until recently it has been the attitude of biochemists that bone crystals are exposed directly to extracellular fluids. It seems not illogical to view bone tissue as protected by a membrane, just as is the tissue of any other system; and for the foregoing reasons it would appear that such a living barrier must exist. When Dr. Edwards A. Park was confronted with this suggestion, he at once recalled that for many years he has used the term "periosteum of the trabecula" for the layer of osteoblasts which apparently completely surrounds the trabecula like a sausage casing. From his collection he pulled several slides (Fig. 5, 6 and 7) showing evidence of an actual "mechanical" membrane.

In this sketchy and sometimes highly speculative synopsis of bone metabolism and calcium homeostasis in the human body, there is undoubtedly much that will be altered as knowledge accrues. Yet for the clinician it offers a theoretical concept upon which to attempt diagnosis on the basis of functional alterations, and hence a physiologic approach to therapy. In light of these principles, it may be well to examine briefly certain of the conditions which may be observed in association with hypercalcemia and hypocalcemia. According to our expressed theoretical concepts, whenever hyper- or hypocalcemia exists there must be an abnormality intrinsic in the bone itself or an abnormality of one or more of the forces which influ-

FIG. 5. Rib of a marasmic infant showing complete cessation of new bone formation. At first glance one might think that the endosteal membrane had disappeared, but the existence of a complete membrane comes to light as the result of avulsion occurring as an artefact in the process of cutting (**a**). Such artefacts are frequent and reveal beautifully the existence of an endosteal complete membrane, otherwise unsuspected.

ence the chemical exchange at the interfaces of bone and extracellular fluid, or both. There may even be cases in which one process predominates in a certain area and the other process predominates in a different area.

ABNORMAL PHYSICAL STATES

There are instances in which physical alterations can overcome the homeostatic mechanisms. These are seen more commonly by the pediatrician than the internist, probably because newly laid down bone has a greater surface area for deposition and resorption, and the crystalline salts are smaller and better hydrated (13). For example, in widespread poliomyelitis or in fracture necessitating huge areas to be immobilized in casts, there has been seen hypercalcemia resulting in uremia—the whole lethal state reversible when mobilization was instituted with cessation or diminution of the rapid atrophy of disuse (9b). We view in broadly similar fashion the hypercalcemia not infrequently seen in widespread osteolytic cancers (46), influenced beneficially by those steroids which, temporarily at least,

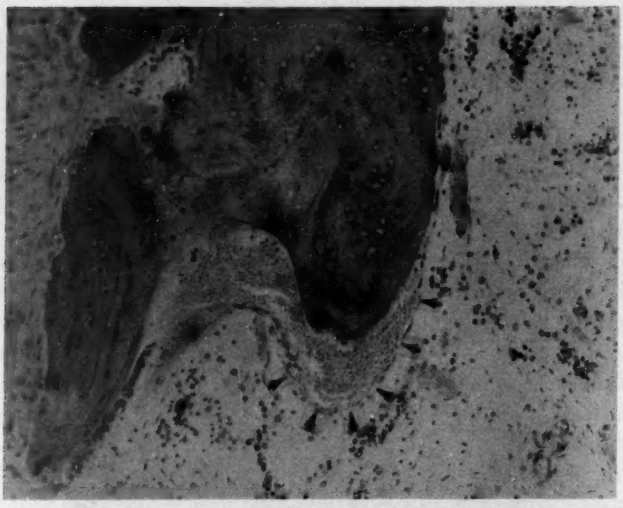

FIG. 6

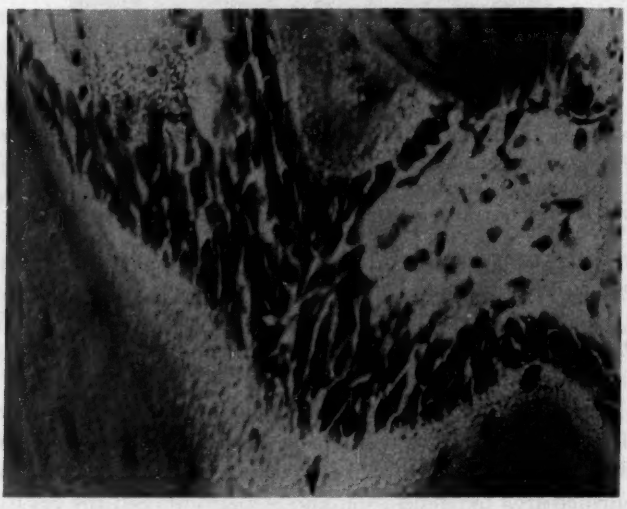

FIG. 7

FIG. 6. Section of scorbutic bone with hemorrhage under the "periosteum of the trabecula." The red cells are contained within the layer of osteoblasts, demonstrating clearly the presence of an endosteal membrane (indicated by arrows).

FIG. 7. Section taken tangentially through a covering layer of osteoblasts (such as seen cut transversely in Figure 2), showing how completely the surface of a trabecula is covered by the carpet of osteoblasts in the enveloping endosteal membrane. (Courtesy of Dr. E. A. Park.)

impede the cellular activity of the carcinoma, and are often made worse by the opposite type of sex hormone. We have long felt that knowledge as to why and how one cancer cell placed adjacent to an osteoblast induces the osteoblast to melt away its matrix, whereas another cancer cell stimulates deposition of new bone, would be of inestimable value. We have never been able to devise an experimental approach to the problem.

There are other types of abnormal tissue which occasionally induce hypercalcemia, apparently by a remote, and hence blood-borne mechanism. We have now seen 5 patients with lung cancer and several with cancer of the bladder, breast and esophagus, who had hypercalcemia but no radiologic or postmortem evidence of skeletal metastases (47). In 2 of these there was prompt restoration to normocalcemia when the primary neoplasm was removed, only to have hypercalcemia recur when local or metastatic relapse became evident. Recently this set of circumstances has been reported in 10 instances from another clinic (48).

Effects of adrenal steroids and of vitamin D. Sarcoid frequently (49), and certain other granulomas occasionally (46), are also associated with hypercalcemia which cannot be accounted for by visible lesions in the bones. In these cases, adrenal steroids in some amazing way almost invariably restore the serum calcium level to normal (50, 51)—as occurs often, though less uniformly in metastatic cancer (52). Toxic doses of vitamin D also produce hypercalcemia, more pronounced with a high calcium intake. In the rat this will take place even in the absence of calcium in the diet, and will result in destruction of the skeleton. In the human subject, cortisone will block the mechanism of hypercalcemia and promptly restore the serum calcium level to normal (52). However, the effects are not completely dissipated, for excessive urinary excretion of calcium continues even after cortisone has restored normocalcemia (29).

The mechanism whereby cortisone affects bone remains, to me at least, a complete enigma. There can be no doubt that in many patients with spontaneously occurring Cushing's syndrome and in many persons receiving large doses of adrenal steroids dangerous degrees of osteoporosis develop. However, there is no evidence as yet as to the means by which these effects have been brought about; under the microscope the bone units look simply small and thin, as in senility. Very puzzling also is the fact that adrenal insufficiency is sometimes associated with hypercalcemia (53–56).

Hyperparathyroidism and hypoparathyroidism can be reasonably well visualized in the light of what has been said. Yet it is perhaps a piece of information, useful for the clinician to know, that in some 10 cases, adrenal steroids have not reduced the hypercalcemia of hyperparathyroidism—a procedure which may prove therefore valuable in differential diagnosis (52, 57).

Other than in hypoparathyroidism, and transiently in acute pancreatitis (58), hypocalcemia is not met except in serious bone disorders, and then usually with concomitant vitamin D deficiency. Observations on the absence of tetany when sprue is seen in Puerto Rico and the increasing likelihood of tetany as sprue is met farther north fit well with the experimental data of Elizabeth Harrison, who found that hypocalcemic tetany quickly occurred as a result of starvation in rachitic rats deprived of vitamin D but not in rats given the same rachitogenic diets with small additions of vitamin D (16). It is hard to escape the belief that vitamin D has some intrinsic importance to bone metabolism itself, in addition to its obvious effect on calcium absorption from the gut.

There are many other skeletal problems of great interest to the pediatrician and internist. Rickets is now most commonly seen here in association with phosphate-losing kidneys, resulting in a continuously low level of serum phosphorus and an environment in which it is impossible for the bone cell to lay down its lime salt. Vitamin D deficiency is relatively rare along the Eastern seaboard. Hypophosphatasia with its attendant severe rickets brings up other interesting problems, for we had about concluded that alkaline phosphatase is present in bones as an energy device and not as a part of the machinery for laying down crystal. One might also speculate on the curious syndrome produced by excessive doses of milk and alkali together. Like vitamin D poisoning, it is reversible if the treatment is stopped in time, though the effect may be cumulative and the hypercalcemia may not disappear for many months after both milk and alkali have been stopped (59). The so-called idiopathic hypercalcemia of infants (60, 61), seen more in Britain than in the United States so far, resembles in many ways D intoxication—either from an exaggerated effect of D or failure of some mechanism for its destruction. This question should soon be answered when bioassays of serum from such infants have been carried out.

There are thus many fascinating vistas for investigation in this field, at present but slightly tilled.

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Letter to the Editor

THE INTRAVENOUS USE OF SODIUM TOLBUTAMIDE IN ACUTE STUDIES

To the Editor:

The recent surge of interest in the sulfonylurea compounds as hypoglycemic agents in man has stimulated a search for explanations of their mechanism of action. Considerable clinical information bearing on this problem has already been obtained with orally administered tolbutamide (Orinase) or carbutamide (BZ55). It seemed likely that additional valuable, and possibly more precise, information as to the mechanisms involved in sulfonylurea-induced hypoglycemia in man might be obtained if these compounds could be administered as single rapid doses by a method similar to that routinely employed in standardized intravenous administration of insulin. The object of acute studies would be to produce metabolic effects comparable to those observed following intravenous insulin with respect to time and degree of effect, and to determine the part played by other factors such as the endocrine system in the total response. The use of intravenous tolbutamide has been reported as a screening test for the responsiveness of diabetic patients and for other investigative purposes, but the infusion was usually given slowly or in relatively small dosage (1, 2).

MATERIALS AND METHODS

Sodium tolbutamide (sodium, 1-butyl-3-p-tolysulfonylurea) (U 7064) was provided as a sterile powder in bottles containing 1 gram. It is highly soluble in isotonic sodium chloride, which was added just prior to administration, to yield a final concentration of 10 per cent and a pH 9.1. The solution was clear and colorless. The period of intravenous injection lasted three minutes. If the material was given in less time, considerable local discomfort occurred and there was a burning sensation along the course of the vein; in addition, there was occasionally a mild systemic effect consisting of headache and a general feeling of warmth. With the three-minute standard injection period employed in our studies no significant undesirable effects were observed. However, until more is known about the toxicity of tolbutamide the customary precautions regarding dosage and administration should be rigidly observed.

RESULTS

Sodium tolbutamide was given in dosages ranging from 13 mg. to 42 mg. per Kg. of body weight (average dose: 25 mg. per Kg.) (Table 1). In nor-

TABLE 1. BLOOD GLUCOSE LEVEL AFTER INTRAVENOUS SODIUM TOLBUTAMIDE

Subject	Dose of sodium tolbutamide (mg./Kg. body wt.)	Blood glucose level (% fall in 30 min.)
L.C. (normal)	24.7	46
	37.0	45
S.D. (normal)	18.7	51
	25.4	45
R.S. (normal)	23.0	41
	31.0	46
	38.0	63
W.W. (normal)	20.3	58
	20.3	59
N.M. (normal)	39.6	64
W.Y. (normal)	13.0	37
F.H. (diabetes mellitus)	25.0	37
N.N. (diabetes mellitus)	41.0	2
C.M. (diabetes mellitus)	42.0	1

mal subjects the blood glucose level fell promptly, maximum hypoglycemia usually being attained in thirty minutes (Fig. 1), and the reduction ranging between 37 and 64 per cent. In some instances the nadir was not reached until forty or forty-five minutes (Subject L.C., Fig. 1). The degree of hypoglycemia varied inconsistently directly with the dosage of sulfonylurea. In cases of unstable ("juvenile") diabetes mellitus (Subjects N.N., C.M., Table 1) there was no significant hypoglycemia after tolbutamide, whereas in cases with onset during maturity there was significant hypoglycemia (Subject F.H., Table 1). In contrast to the standardization of the dosage of intravenous insulin (0.1 unit per Kg. of body weight), we were unable to establish a standard intravenous dosage of sodium tolbutamide which would produce the same degree of hypoglycemia in all subjects. It was necessary, therefore, to gauge the dose of intravenous sulfonylurea by the effect obtained with intravenous insulin (0.1 unit per Kg., or other dosages) in order to establish the amount required to produce an equivalent hypoglycemic response. In general, the amount of sodium tolbutamide required was at least 18–20 mg. per Kg. of body weight, but was often more.

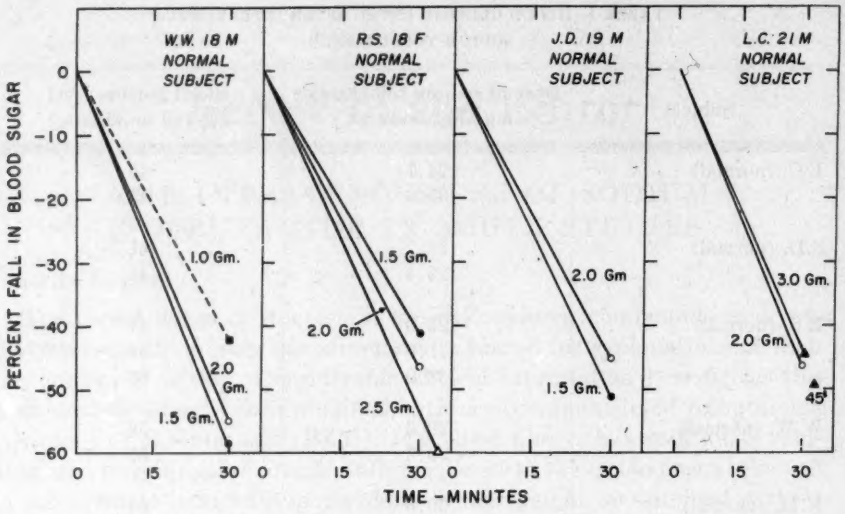

FIG. 1. The time at which maximum hypoglycemia occurred, and the percentile fall in the blood sugar level, in 4 normal subjects. The dose of sodium tolbutamide given at zero time is indicated for each test.

In preliminary studies, no tachyphylaxis was observed with this compound. Once a subject's hypoglycemic dose is established with sodium tolbutamide, it may be used in subsequent studies in which reproducible hypoglycemic effects are desired. Measurements of blood levels of tolbutamide have shown that the material is rapidly excreted or metabolized and that only negligible amounts remain in the blood after twenty-four hours (3). Therefore, studies can be repeated at about 48-hour intervals without serious concern as to overlapping effects.

With the use of intravenous sodium tolbutamide, it has been possible to

TABLE 2. PLASMA HYDROCORTISONE LEVEL AFTER INTRAVENOUS SODIUM TOLBUTAMIDE

	Plasma hydrocortisone (μg./100 ml.)*						
	Control		10 min.	30 min.	60 min.	120 min.	180 min.
N.M. (normal)	9.4	*Sodium tolbutamide, 2.5 Gm. i.v.*	12.2	14.7	18.1	31.9	12.4
N.N. (diabetes mellitus)	20.3		17.7	17.7	13.9	22.7	22.7

* Normal level: 6–25 μg./100 ml. (4).

perform a variety of acute metabolic and endocrine studies in both normal and diseased subjects. For instance, results of intravenous insulin tolerance tests, insulin-glucose tolerance tests, glucose-insulin tolerance tests and measurements of rate of disappearance of infused glucose have been compared with results of the same test procedures using sodium tolbutamide instead of insulin (*e.g.*, intravenous tolbutamide tolerance tests, and tolbutamide-glucose tolerance tests). Intravenous sodium tolbutamide has been found useful in studies comparing the effect of insulin with that of tolbutamide on the volume of distribution of the so-called "insulin-responsive" pentoses, *l*-arabinose and *d*-xylose (3). The acute effects of tolbutamide on adrenocortical function have also been determined (Table 2) and found not to be significant. Studies of acute effects on other endocrine glands such as the adrenal medulla and thyroid are in progress.

THOMAS F. FRAWLEY, M.D.*

National Institute of Arthritis and Metabolic Diseases,
National Institutes of Health,
Bethesda, Maryland.

* Present address: Department of Endocrinology and Metabolism, Albany Medical College, Albany, N. Y.

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The Endocrine Society

THE 1958 ANNUAL MEETING

The Fortieth Annual Meeting of The Endocrine Society will be held in the St. Francis Hotel, San Francisco, California, Thursday, Friday and Saturday, *June 19, 20 and 21, 1958.*

THE 1958 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955, Dr. Frederick L. Hisaw in 1956, and Dr. Joseph C. Aub in 1957.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

NINTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE MEDICAL COLLEGE OF GEORGIA

Augusta, Georgia, October 21-25, 1957

The faculty will consist of 24 eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the Assembly.

For further information concerning the program and registration, write to *Dr. Robert B. Greenblatt, Department of Endocrinology, Medical College of Georgia, Augusta, Georgia.* Registration is limited to 100; the tuition fee is $100.00. Rooms will be reserved for the students and faculty at the Bon Air Hotel. Residents and fellows will be admitted for $35.00.

(*For Faculty and Program, see June issue*)

THE ACADEMY OF PSYCHOSOMATIC MEDICINE

The program of the Fourth Annual Meeting of The Academy of Psychosomatic Medicine, to be held *October 17–19, 1957* at the Morrison Hotel, Chicago, Illinois, will be devoted to "Psychosomatic Aspects of Obstetrics, Gynecology, Endocrinology and Diseases of Metabolism." The meeting will be open to all scientific disciplines, as well as to psychologists, social workers and nurses. Information may be obtained from Dr. William S. Kroger, Secretary, 104 South Michigan Avenue, Chicago 3, Illinois.